INDEX NUMBER

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Clinical and Experimental

A "New" Antibody, Anti-Rh^c, Resulting From Isosensitization by Pregnancy With Special Reference to the Heredity of a New Rh-Hr Agglutinogen Rh_2^c. Lester J. Unger, M.D., and Alexander S. Wiener, M.D., New York, N. Y. ---------- 835

Changes in Serum Proteins, Lipoproteins, and Protein-Bound Carbohydrates in Relation to Pathologic Alterations in the Microcirculation of Diabetic Subjects. Jørn Ditzel, M.D., and Pierre Moinat, M.D., Boston, Mass. ---------- 843

Cobalt as a Producer of Porphyrinuria and Polycythemia. Jouko Saikkonen, M.D., Helsinki, Finland ---------- 860

Erythrocyte Electrolytes in Muscle Disease. Robert M. Dowben, M.D., and Kenneth R. Holley, Chicago, Ill. ---------- 867

Studies on the Normal Serum Panagglutinin Active Against Trypsinated Human Erythrocytes. V. The Nonidentity of the Agglutinin With Serum Trypsin Inhibitor and the Components of Complement. Kenneth J. Coffield, M.D., and Theodore H. Spaet, M.D., New York, N. Y. ---------- 871

The Effect of Synthetic Angiotonin Upon the Heart of the Intact Dog. G. M. Maxwell, M.D., M.R.C.P., C. A. Castillo, M.D., C. W. Crumpton, M.D., J. E. Clifford, M.D., and G. G. Rowe, M.D., Madison, Wis. ---------- 876

Cranberry Juice and the Antibacterial Action of Hippuric Acid. Phillis T. Bodel, M.D., Ramzi Cotran, M.D., and Edward H. Kass, M.D., Ph.D., Boston, Mass. ---------- 881

Airway Resistance Studies in Bronchial Asthma. William E. Ruth, M.D., and Charles E. Andrews, M.D., Kansas City, Kan., and Kansas City, Mo. ---------- 889

Laboratory Methods

A Hydraulic Pressure Generator for Testing the Dynamic Characteristics of Blood Pressure Manometers. Frank W. Noble, M.E.E., Bethesda, Md. ---------- 897

The Enzymatic Spectrophotometric Method for Determination of Uric Acid. Lois Liddle, B.S., J. E. Seegmiller, M.D., and Leonard Laster, M.D., Bethesda, Md. ---- 903

(Contents continued on inside front cover)

PUBLISHED BY THE C. V. MOSBY COMPANY, ST. LOUIS 3, MISSOURI

Contents—*(Continued from front cover)*

Proceedings of the Thirty-Second Annual Meeting of the Central Society for Clinical Research

ABSTRACTS

The Significance of Amino Acid Transport in Protein Synthesis. David M. Kipnis, M.D., Paulo A. Ayroza Galvao, M.D., Glenn Greene, M.D., and William H. Daughaday, M.D., St. Louis, Mo. ---------- 914

Salt Loading and Exchangeable Electrolytes in Patients Without and With Mild Hypertension. Walter M. Kirkendall, M.D., Richard D. Peterson, M.D., and Mark L. Armstrong, M.D., Iowa City, Iowa ---------- 915

Erythropoietic Activity of Plasma in Polycythemia. Donald R. Korst, M.D., Barbara E. Whalley, Ph.D., and Frank H. Bethell, M.D., Ann Arbor, Mich. ---------- 916

Evidence for a Direct Renal Tubular Action of Parathyroid Extract. A. R. Lavender, M.D., Impi Aho, B.A., Howard Rasmussen, M.D., and Theodore N. Pullman, M.D., Chicago, Ill. ---------- 916

Nonperistaltic Esophageal Motility Accompanying Experimentally Produced Heartburn. Harold P. Lazar, M.D., Ernesto J. Puletti, M.D., William W. Douglas, Stuart Danovitch, and E. Clinton Texter, Jr., M.D., Chicago, Ill. ---------- 917

Reversal of the Diurnal Urinary Excretion of Water and Electrolytes as a Manifestation of Primary Hyperaldosteronism. Edward J. Lennon, M.D., Phillip P. Ruetz, M.D., and William W. Engstrom, M.D., Milwaukee, Wis. ---------- 918

The Confusion of Distribution and Diffusion. Benjamin M. Lewis, M.D., E. J. Hayford-Welsing, M.D., and John D. McGinty, M.D., Detroit, Mich. ---------- 918

Effect of Ethanol on Fatty Acid Metabolism in Vitro. Charles S. Lieber, M.D., Leonore M. DeCarli, B.A., and Rudi Schmid, M.D., Boston, Mass. ---------- 919

The in Vitro Thyroid Response to Propylthiouracil. Robert E. Mack, M.D., Kathleen T. Hart, Ph.D., Dea Druet, M.S., and Mary Anne Bauer, B.S., St. Louis, Mo. ---- 920

Evidence for a Direct and Immediate Effect of Insulin on Hepatic Glucose Output in Diabetic and Normal Dogs. Leonard L. Madison, M.D., Burton Combes, M.D., Reuben Adams, M.D., and William Strickland, M.D., Dallas, Texas ---------- 920

(Contents continued on page 40 of advertisements)

(Editorial and Business Communications on page 26)

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Babson, A. L., Read, P. A., and Phillips, G. E.: The importance of the substrate in assays of acid phosphatase in serum. Am. J. of Clin. Path. 32:1 83-87, July 1959.

A comparison is made between six generally used substrates for their relative specificity to prostatic and erythrocytic acid phosphatase. Results showed that a new substrate, alpha-naphthyl phosphate, was twice as specific as beta-glycerophosphate and 40-100 times as specific as all other substrates investigated. Acid phosphatase assays in serum should be specific for that enzyme arising from cancerous prostatic tissue. The substrate alpha-naphthyl phosphate* is specific for this enzyme.

Babson, A. L., and Read, P. A.: A new assay for prostatic acid phosphatase in serum. Am. J. Clin. Path. 32:1, 88-91, July 1959.

A new and specific method for the determination of prostatic acid phosphatase in serum is presented. The method is based on the use of a new substrate alpha-naphthyl phosphate which measures only prostatic acid phosphatase. The method is simple and rapid to perform with a minimum of manipulation. In this procedure the color developed represents prostatic enzyme activity alone. The reagents for this new method are available in convenient and stable form.*

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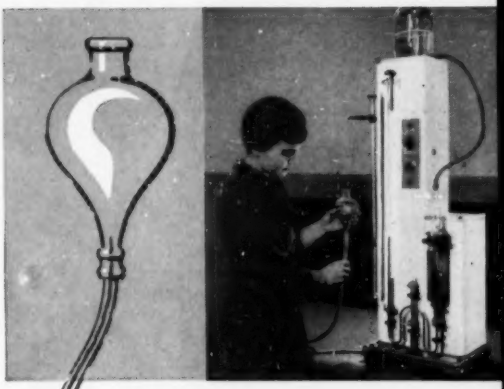

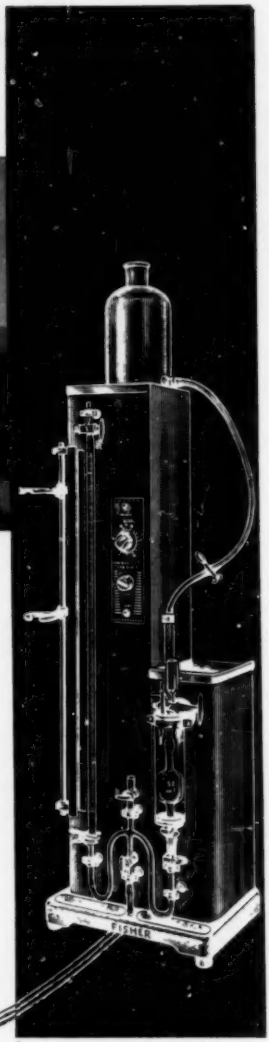

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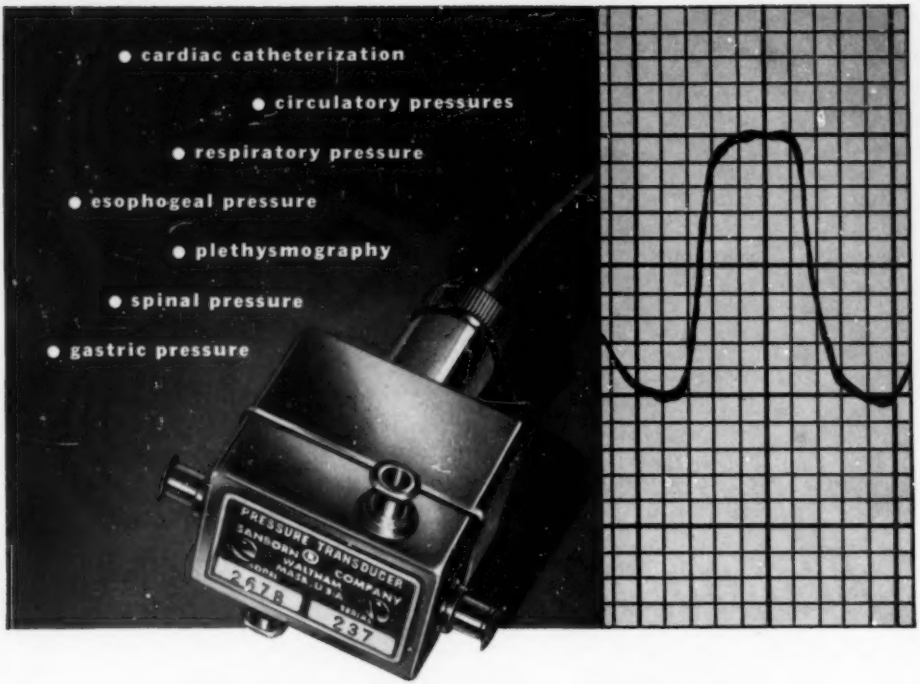

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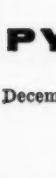

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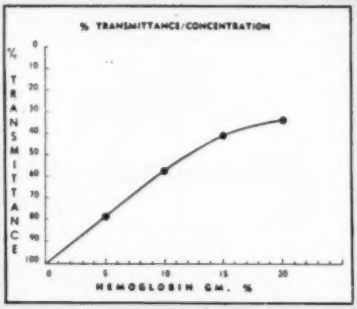

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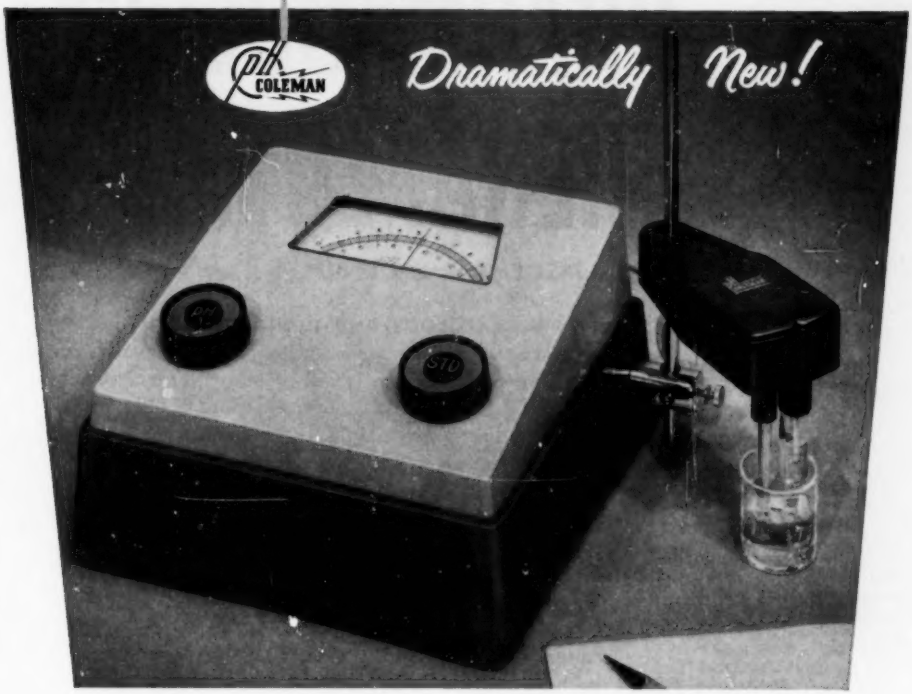

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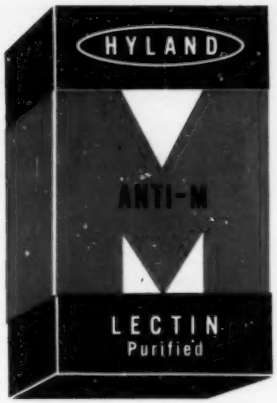

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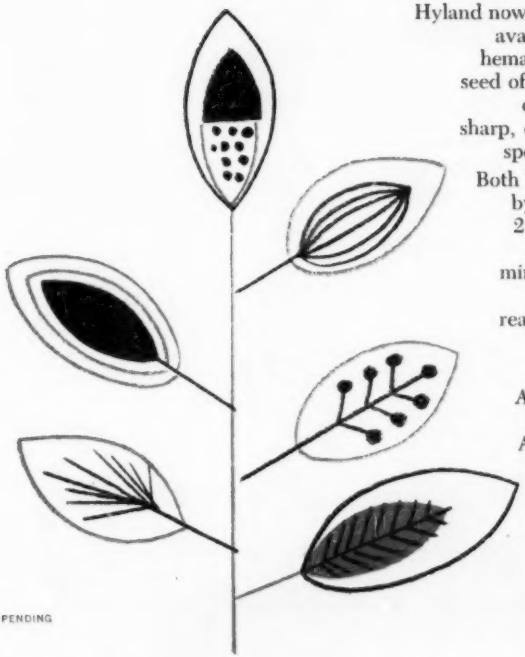

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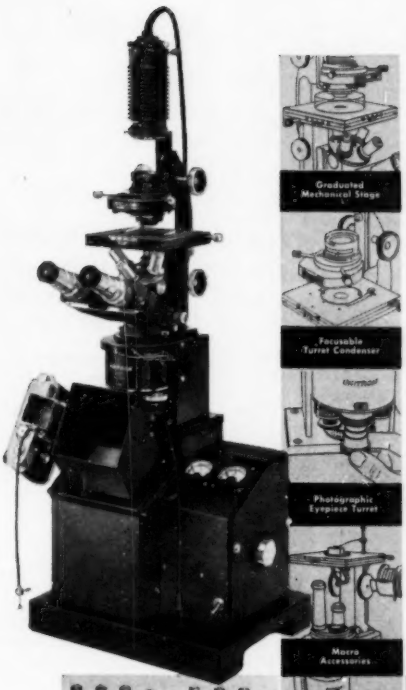

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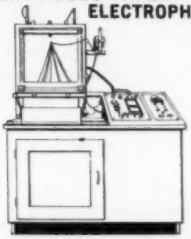

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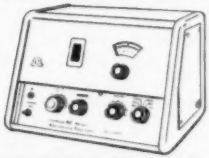

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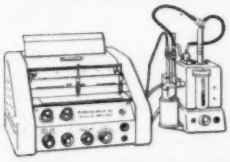

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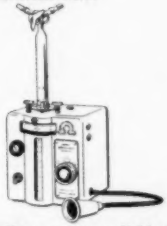

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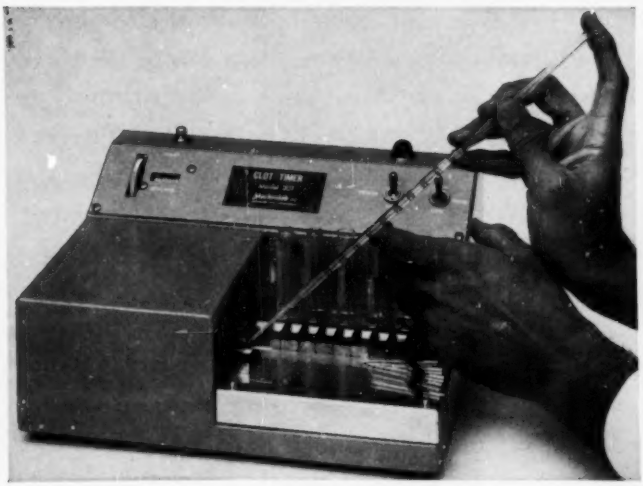

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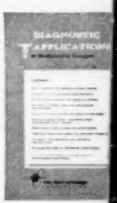

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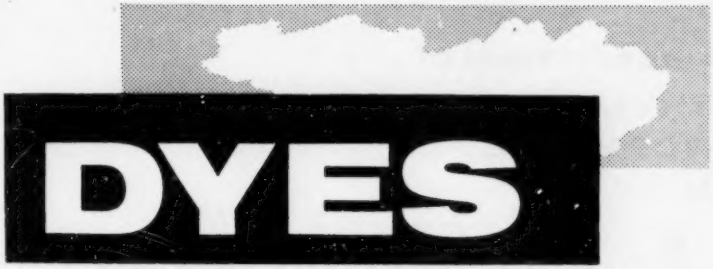

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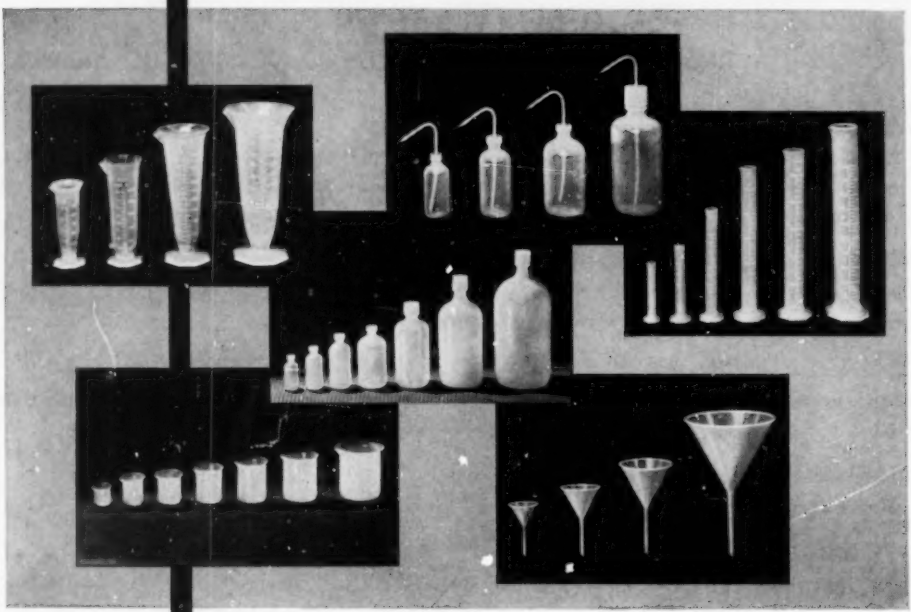

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The Journal of Laboratory and Clinical Medicine

VOL. 54 DECEMBER, 1959 NO. 6

CLINICAL AND EXPERIMENTAL

A "NEW" ANTIBODY, ANTI-**Rh**^{C}, RESULTING FROM ISOSENSITIZATION BY PREGNANCY WITH SPECIAL REFERENCE TO THE HEREDITY OF A NEW Rh-Hr AGGLUTINOGEN $\mathfrak{Rh}_2^c$

LESTER J. UNGER, M.D.,* AND ALEXANDER S. WIENER, M.D.,**
NEW YORK, N. Y.

A STRIKING recent development regarding the Rh-Hr types was the observation that certain rare **Rh**$_0$-positive (or $\mathfrak{Rh}_0$-positive) individuals can be isosensitized by **Rh**$_0$-positive blood and produce antibodies closely resembling anti-**Rh**$_0$ in specificity.† This seeming paradox has been explained by the demonstration that in Rh agglutinogens‡ the **Rh**$_0$ factor has associated with it many other blood factors which we have designated **Rh**^{A}, **Rh**^{B}, **Rh**^{C}, **Rh**^{D}, etc. In certain rare **Rh**$_0$-positive individuals the Rh agglutinogen may lack one or more of these associated factors, and such individuals can be sensitized by "standard" Rh-positive blood. The antibody produced, anti-**Rh**^{A} or anti-**Rh**^{B} or anti-**Rh**^{C}, etc., as the case may be, is indistinguishable from ordinary anti-**Rh**$_0$ in specificity except that it fails to react with the **Rh**$_0$-positive blood of the individual who has produced the antibody as well as certain other rare **Rh**$_0$-positive bloods. The first case of this nature was reported by Shapiro,[1] but the serum of his patient is no longer available for study. In

From the Blood Bank, New York University-Bellevue Medical Center, and Office of the Chief Medical Examiner, New York, N. Y.

Received for publication April 9, 1959.

*Director, Blood and Plasma Bank, New York University-Bellevue Medical Center.

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†In conformity with the recommendation of the Committee on Medicolegal Problems of the American Medical Association, in this paper, symbols for phenotypes, agglutinogens, and blood group systems are printed in regular lightface type, symbols for blood factors and their specific antibodies in boldface type, and symbols for genes and genotypes are printed in italics.

‡Rh agglutinogens are defined as comprising all those agglutinogens of the Rh-Hr blood group system which have in common the blood factors **Rh**$_0$, namely, agglutinogens Rh$_0$, Rh$_1$, Rh$_1^{\gamma}$, Rh$_2$, etc.

the cases more recently encountered, comparison of the patients' sera with one another showed that the antibodies all differed in specificity. A complete review of the literature has been given in previous papers[2-7] and will not be repeated here. Wiener[8a, b] assigned the symbol anti-**Rh**^{A} to the antibody in the serum of a mother who gave birth to an erythroblastotic infant. Unger and Wiener[6] identified a new antibody in the serum of an **Rh**$_0$-positive patient who had had a hemolytic transfusion reaction, and to it they assigned the symbol anti-**Rh**^{B}. The purpose of this present paper is to describe still another "new" antibody stimulated by pregnancy, to which we have assigned the symbol anti-**Rh**^{C}.

With the aid of antisera anti-**Rh**^{A}, anti-**Rh**^{B}, and anti-**Rh**^{C}, blood types such as Rh_0, Rh_1, Rh_2, etc., can be subdivided, since, as has been pointed out, while "standard" **Rh**$_0$-positive blood has all the blood factors **Rh**^{A}, **Rh**^{B}, **Rh**^{C}, etc., as well as **Rh**$_0$, rare Rh-positive bloods exist lacking one or more of these blood factors. Theoretically, there are 8 possible Rh agglutinogens definable with sera anti-**Rh**^{A}, anti-**Rh**^{B}, and anti-**Rh**^{C} when the **Rh**$_0$ blood factor is "standard," and to date we have encountered 5 of these. There are 8 additional possible agglutinogens where the **Rh**$_0$ blood factor is a variant, and we have already identified all 8 of these.[7] The exceptional blood types differentiated by the absence of one or more of the factors have added to their phenotype symbols superscript small letters a, b, or c, etc., to indicate which of these factors is missing; e.g., type Rh_1^a blood differs from "standard" type Rh_1 blood only in its failure to react with anti-**Rh**^{A} serum. Similarly, blood of type Rh_1^b resembles standard type Rh_1 blood except for its failure to react with anti-**Rh**^{B} serum; type Rh_1^{ab} resembles standard type Rh_1 blood except for its failure to react with both anti-**Rh**^{A} and anti-**Rh**^{B}, etc. The presumption is that each of the new rare Rh-Hr agglutinogens defined in this way are inherited by corresponding allelic genes (Wiener's theory of multiple allelic genes). Unfortunately, the type Rh_1^a mother in the case of Wiener and Geiger had a type Rh_1 husband and none of her type Rh_1 children lacked the **Rh**^{A} factor. Similarly, a family study by Unger and Wiener and Weiner, of their patient sensitized to the **Rh**^{B} factor, failed to yield any evidence throwing light on this problem. Unger and Wiener did succeed, however, in demonstrating the presence of the rare agglutinogen Rh_0^a in a mother and child.[4, 5] In the present case we have been more fortunate and, as will be shown, the patient's family pedigree provides convincing evidence that the rare agglutinogen Rh_2^c is transmitted by means of a corresponding allelic gene R^{zc}.

METHODS

The following are our routine procedures. As soon as pregnancy is diagnosed, a specimen of the patient's blood is sent to the blood bank for determination of the A-B-O group, M-N type and Rh-Hr type. Sensitivity tests are also performed on her serum to detect atypical antibodies.[9, 10] If her blood is Rh-negative* the husband's blood is examined to determine his A-B-O blood group, M-N type, and Rh-Hr type. If the husband

*Recently, Rh-positive expectant mothers are subjected to the same routine blood examinations as Rh-negative expectant mothers, and as a result of this routine, the present case was detected.

is Rh-positive, the Rh-negative expectant mother's blood is examined at monthly intervals for atypical antibodies until the eighth month of pregnancy, then twice during that month and thereafter at weekly intervals until term. This schedule is possible, of course, only if the patient cooperates. If the patient is Rh-positive and the tests for sensitivity show the presence of atypical antibodies, the schedule for antibody examinations is the same as for Rh-negative mothers. If, however, no atypical antibodies are found in a Rh-positive expectant mother, no further sensitivity tests are carried out until just before admission to the hospital. In addition, at this time, if the patient is group O and her husband is group A, B, or AB, the mother's serum is titrated for anti-**A** and anti-**B**. If, for any reason, the patient unexpectedly enters the hospital without this final blood examination, whenever possible the titrations are carried out immediately after admission. A direct antihuman globulin test is done routinely on the cord blood of every newborn infant. The details of the techniques used for blood typing and antibody titrations have already been given in previous publications.[3, 9-13]

CASE HISTORY

Mrs. M. A., 23 years of age, was born in the United States although her parents were born in the Dominican Republic. She is of mixed ancestry, namely, Spanish and American Indian. Except for asthma, from which she has been suffering for a number of years, she has always been well. She has no history of previous blood transfusions. She had two children, neither of whom was jaundiced at birth, and both are living and well at the present time. She has had no miscarriages. The patient was pregnant for the third time when she reported to the outpatient department, and 4 months later was delivered of twin boys. She was discharged from the hospital 6 days postpartum.

RESULTS

Serologic Studies.—At the time of the first serologic examination during the pregnancy, the expectant mother was found to be group O, type N, type $\mathfrak{R}h_2rh$. The patient's red cells were not agglutinable in saline medium by bivalent anti-**Rh$_0$** serum, although they were agglutinable by all univalent anti-**Rh$_0$** sera tried and to about the same titer as standard Rh-positive cells, so that the cells contained a high-grade **Rh$_0$** variant. Her blood cells were also strongly clumped by anti-**Rh^A** and anti-**Rh^B** sera. The patient's cells were positive for blood factors **F**, **k**, **U**, **Jk^a**, **P**, and negative for **K**, **S**, **Le^a** and **He**. Although tests for isosensitization were negative at first (see Table I, 5-14-58), approximately 2½ months later Rh antibodies were found in the patient's serum (Table I, 7-26-58), but then only in a titer of 4 units and demonstrable only by the ficinated-cell and ficinated-cell antiglobulin methods. The latter method was first described by one of us (L. J. U.).[9-11] As Table I shows, the titers of the Rh antibody in her serum rose as the pregnancy progressed, and sensitization then became demonstrable by all 5 methods of titration used. The antibodies in the serum resembled anti-**Rh$_0$** in specificity except for failure to clump the patient's own cells. Since the antibody was shown to be different from anti-**Rh^A** and anti-**Rh^B**, we have assigned to it the symbol anti-**Rh^C**.

The patient gave birth to a pair of dissimilar twin boys. There were two placentae. Examination of the blood of twin A showed a hemoglobin concentration of 18.4 Gm. (127 per cent). His blood groups were O MN

TABLE I. CHANGES IN TITERS OF THE ANTIBODY (ANTI-**Rh**^{C}) IN THE PATIENT'S SERUM DURING PREGNANCY

DATE	METHODS OF TITRATION (TITERS IN UNITS*)				
	SALINE AGGLUTINATION	ALBUMIN-PLASMA CONGLUTINATION	ANTIGLOBULIN	FICINATED CELL	FICINATED-CELL ANTIGLOBULIN
5-14-58	0	0	0	0	0
7-26-58	0	0	0	4	4
8-7-58	2	4	0	16	16
8-13-58	1	4	2	16	16
9-4-58	4	4	16	32	128
9-12-58†	2	4	4	64	64

*The titer value is the reciprocal of the highest dilution of the serum giving a one plus (+) reaction. In each case two sets of group O, Rh-positive cells (type Rh_1Rh_1 and Rh_2rh) were used and the titer values obtained averaged. Type rh′, rh″, and rh cells were used as controls, and in each titration the serum failed to clump these cells.

†On Sept. 15, 1958, the patient gave birth to twins.

Rh_1rh. The direct antiglobulin reaction* was two plus (2+) positive, and the cord bilirubin concentration was 3.0 mg. per 100 ml. The liver and spleen were not palpable but slight jaundice appeared 48 hours after birth. Examination of the blood of twin B showed a hemoglobin concentration of 24 Gm. (160 per cent). The blood groups were O N Rh_2Rh_0. The direct antiglobulin reaction was two plus (2+) positive and the cord bilirubin concentration was also 3.0 mg. per 100 ml. In this twin also, the liver and spleen were not palpable, and slight jaundice appeared 48 hours after birth. Thus, both infants exhibited signs of erythroblastosis fetalis, but because of the mildness of the manifestations, treatment by exchange transfusion was not indicated, and at no time did the need arise for simple transfusion.

TABLE II. ILLUSTRATIVE REACTIONS OF THE SERA FROM THREE SENSITIZED **Rh**$_0$-POSITIVE PATIENTS

CELLS	ANTISERA			
	ANTI-**Rh**$_0$*	ANTI-**Rh**^{A} MRS. S.	ANTI-**Rh**^{B} MRS. V.	ANTI-**Rh**^{C} MRS. A.
$Rh_1^{ab}rh$ Mrs. S.	+	0	0	+
$Rh_2^{b}rh$ Mrs. V.	+	+	0	+
$Rh_2^{c}rh$ Mrs. A.	+	+	+	0
Rh_0^{ac} (64950)†	+	0	+	0
"Standard" Rh blood‡	+	+	+	+

*Serum from an **Rh**$_0$-negative individual sensitized to blood factor **Rh**$_0$.

†The Rh-positive individual who provided this rare type of cells was not sensitized.

‡Since with rare exceptions **Rh**$_0$-positive blood has all the blood factors, namely, **Rh**^{A}, **Rh**^{B}, **Rh**^{C}, etc., associated with the **Rh**$_0$ blood factor, there is no need to indicate this fact in the symbols for the Rh-Hr phenotype. For the rare **Rh**$_0$-positive bloods lacking one or more of the blood factors **Rh**^{A}, **Rh**^{B}, **Rh**^{C}, a small superscript letter is used corresponding to the missing factor.

As has already been mentioned, results of the examination of the mother's serum show that this case falls into the same category as our two previous cases.[6-8] Table II demonstrates differences in specificities among anti-**Rh**^{A}, anti-**Rh**^{B}, and this patient's serum, anti-**Rh**^{C}. That the antibody in the present

*This test, which is virtually pathognomonic for erythroblastosis fetalis, was first described by one of us (A. S. W.),[15] as well as the direct conglutination and direct blocking tests.[16]

patient's serum is not anti-**Rh**^{A} or anti-**Rh**^{B} follows from the fact that the patient's serum reacts with cells which lack factors **Rh**^{A} and **Rh**^{B} but not with her own cells which contain both factors **Rh**^{A} and **Rh**^{B}. Moreover, the patient's cells react with anti-**Rh**^{A} and anti-**Rh**^{B} to approximately the same titer as "standard" **Rh**$_{o}$-positive cells.

Family Investigation.—To the Rh-Hr type to which the patient belongs may be assigned the symbol $\mathfrak{R}h_2^c rh$. The question whether the agglutinogen $\mathfrak{R}h_2^c$ is hereditarily transmitted by a corresponding allelic gene, like other Rh-Hr agglutinogens (Wiener's theory of multiple alleles)[14, 15] could only be answered by a family investigation. Therefore, blood samples were obtained

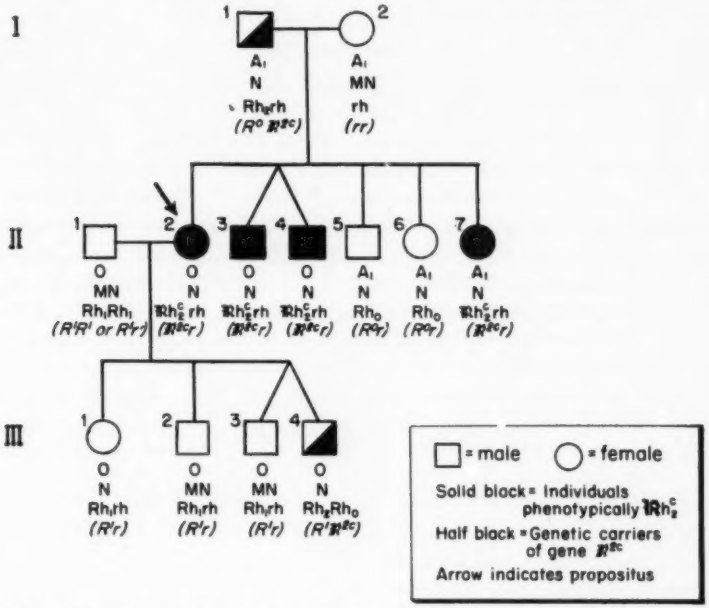

Fig. 1.—Family study showing the hereditary transmission of the rare gene $\mathfrak{R}^{zc}$.

from members of the patient's family and examined for their blood groups, and the results of this pedigree analysis are summarized in Fig. 1.

As shown in that figure, the patient has 2 sisters and 3 brothers; of the latter, two are twins. Her mother is type rh, so that the patient and her 5 siblings all must have received the gene r from that parent. Therefore, the patient who is of type $\mathfrak{R}h_2^c rh$ must be of genotype $\mathfrak{R}^{zc}r$. Of the 5 siblings, the twin brothers and one of the sisters are of type $\mathfrak{R}h_2^c rh$, genotype $\mathfrak{R}^{zc}r$, like the patient, while one brother and one sister are both of type Rh$_o$, genotype $R^o r$. Therefore, the patient's father, who belongs to type Rh$_2$rh, must be a carrier of gene $\mathfrak{R}^{zc}$, genotype $\mathfrak{R}^{zc}R^o$. His blood, however, is agglutinated by the patient's anti-**Rh**^{C} serum, because the gene R^o produces a standard agglutinogen Rh$_o$, reactive with all the antisera for factors associated with factor **Rh**$_o$. Of

the paternal grandparents, only the grandmother was available for testing but she proved to be A_1 MN Rh_0, so that she is not a carrier of gene $\mathfrak{R}^{zc}$. She is not indicated in the pedigree chart, and obviously it must have been the paternal grandfather of the patient who was the source of the $\mathfrak{R}^{zc}$ gene. The patient's husband proved to be type Rh_1Rh_1. Three of the children, including one of the twins, proved to be ordinary type Rh_1rh, genotype R^1r. The other twin, who belongs to type Rh_zRh_0 must be a carrier of gene $\mathfrak{R}^{zc}$, genotype $R^1\mathfrak{R}^{zc}$. Summarizing, therefore, 3 of the patient's 5 siblings belong to the same unusual Rh-Hr type as herself, namely, type $\mathfrak{Rh}^c_2rh$, and the findings indicate that her father and one of her children are carriers of gene $\mathfrak{R}^{zc}$. These results, therefore, support the hypothesis that agglutinogen $\mathfrak{Rh}^c_2$ is determined by a corresponding allelic gene $\mathfrak{R}^{zc}$.

DISCUSSION

Preparations were made anticipating the possibility that this patient might require blood transfusions at the time of labor and that her children might require exchange transfusions. Since the atypical antibody in her serum had been identified as anti-**Rh**^{c} and since blood of type Rh^c is rare, Rh-negative blood was crossmatched and kept available. However, later developments showed that blood was not needed and none was given.

In a previous communication[6] we described a hemolytic posttransfusion reaction in a patient for whom apparently compatible blood was selected using standard crossmatching techniques, namely, the saline agglutination, albumin conglutination, and antiglobulin methods. The results of each of these in vitro tests indicated the blood to be compatible, yet, after administration, hemolytic posttransfusion reactions occurred. On the other hand, sensitivity tests using the ficinated-cell and ficinated-cell antiglobulin methods[9, 10] showed the presence of an atypical antibody in the patient's serum. This caused us to recommend that when atypical antibodies are demonstrable in a patient's serum only with ficinated test cells, additional crossmatching tests be added to the routine, namely, the ficinated-cell crossmatch and the ficinated-cell antiglobulin crossmatch.[6]

It is of interest that the same situation arose with the present patient. If she had required a transfusion at the time the specimen was obtained for the second titration (Table I, 7-26-58), the technician would have crossmatched the patient's blood with blood from an Rh-positive donor. The technician would have justifiably relied on crossmatching tests by the saline agglutination, albumin conglutination, and antiglobulin methods, and would have been unable to detect the incompatibility. Yet the transfusion of such blood could have been followed by a hemolytic reaction. At that time, sensitivity tests only by the ficinated-cell and ficinated-cell antiglobulin methods revealed the presence of atypical antibodies. If, under such circumstances, the additional crossmatch tests recommended by us are performed, the incompatibility would then become apparent. The commonly used method of ficinating red cells requires a considerable quantity of blood. Therefore, the following method for the preparation of ficinated cells devised by Wiener, is recommended.

A 3 per cent suspension of cells in normal saline solution is washed twice with normal saline solution, and the sediment resuspended to the original strength. One drop of this cell suspension is placed in a small test tube (inside diameter 7 to 8 mm.), and to this is added 1 drop of freshly prepared diluted ficin solution.* The mixture is placed in a water bath at 37° C. for 15 minutes. The mixture is then washed once with normal saline solution to stop the action of the enzyme, and the sediment resuspended in one drop of normal saline solution. To this is added a drop of the serum to be tested, and after 1 hour's incubation at body temperature, the reactions are read.

When both donor's cells and patient's cells are ficinated in this fashion, the new crossmatching test we recommend uses the standard routine of the saline agglutination method but with ficinated cells instead of untreated cells (ficinated-cell crossmatch). After the reactions are read, an antiglobulin test can be carried out on the cell sediment (ficinated-cell antiglobulin crossmatch). Simultaneously, tests using patient's serum and patient's ficinated cells as well as donor's serum and donor's ficinated cells can be done as controls. If the results of these tests are negative, an antiglobulin test is done on these sediments (ficinated-cell antiglobulin method).

After the present investigation was completed, we studied two additional cases which provide evidence regarding the mechanism of the new rare varieties of Rh agglutinogens. In one case,[17] a mother and her mildly affected erythroblastotic baby both proved to be type Rh_0, and the blood cells of mother and baby had all 3 factors, **Rh**^{A}, **Rh**^{B}, and **Rh**^{C} as well as **Rh**$_0$, and were clumped to the same titer as standard Rh-positive blood cells by such reagents, including saline agglutinating anti-**Rh**$_0$. Yet the mother's serum contained a potent antibody resembling anti-**Rh**$_0$ in specificity which clumped the baby's cells but not the mother's own cells. This antibody has therefore been assigned the symbol anti-**Rh**^{D}, while the mother's phenotype is assigned the symbol Rh_0^d. Among 7 siblings of the erythroblastotic baby, 2 proved to be type rh and 5 type Rh_0; of the latter all but one were incompatible with the mother's serum. This indicates that the mother and the compatible baby of type Rh_0 are both type Rh_0^d, genotype $R^{od}r$. In the second case,[18] a type Rh_1rh woman who had had a hemolytic transfusion reaction, had a potent Rh antibody in her serum. Her red cells gave typical reactions with anti-**Rh**$_0$ and anti-**Rh**^{A} sera like "standard" Rh_1rh blood, but failed to react with anti-**Rh**^{C} and anti-**Rh**^{D}, and gave weak reactions with anti-**Rh**^{B}. Two siblings of the patient proved also to belong to type Rh_1rh and their blood cells gave reactions identical with those of the patient's blood, while the mother belonged to type Rh_1Rh_1. Thus, patient and both siblings may be classified as type $Rh_1^{\beta cd}$rh, genotype $R^{1\beta cd}r$, while their mother is type Rh_1Rh_1, genotype $R^1R^{1\beta cd}$.

*A 1 per cent stock solution of enzyme is prepared by shaking 100 mg. of ficin in 10 ml. of normal saline solution (or 10 mg. in 1 ml. of normal saline solution). This may be used for periods up to 1 week if stored in the refrigerator. The diluted solution for the test is prepared immediately before it is used by diluting the supernatant solution of the stock suspension with 5 volumes of normal saline solution. Any of this diluted enzyme solution not used immediately is discarded.

SUMMARY

A case is described in which an **Rh**$_0$-positive woman gave birth to fraternal male twins, both **Rh**$_0$-positive, and with signs of mild erythroblastosis fetalis. In the serum of the mother a "new" antibody closely resembling, although different from, anti-**Rh**$_0$ in specificity was found. The patient's red cells had a high-grade **Rh**$_0$ variant and blood factors **Rh**^{A} and **Rh**^{B}, while her serum clumped **Rh**$_0$-positive cells lacking both factor **Rh**^{A} and factor **Rh**^{B}. The antibody in her serum was thus shown to be different from anti-**Rh**^{A} and anti-**Rh**^{B}, and therefore has been assigned the symbol anti-**Rh**^{C}.

The patient's complete Rh-Hr phenotype was $\mathfrak{R}h_2^c rh$, and among her siblings 3 were found to be of this Rh-Hr type, while 2 were of type Rh$_0$. These results and the findings on the patient's parents and children provide evidence supporting the theory that the agglutinogen $\mathfrak{R}h_2^c$ is determined by a corresponding allelic gene $\mathfrak{R}^{zc}$ in analogy to the other already known Rh-Hr agglutinogens.

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CHANGES IN SERUM PROTEINS, LIPOPROTEINS, AND PROTEIN-BOUND CARBOHYDRATES IN RELATION TO PATHOLOGIC ALTERATIONS IN THE MICROCIRCULATION OF DIABETIC SUBJECTS

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RECENT studies have demonstrated that changes occur in the serum proteins, lipoproteins, and protein-bound carbohydrates of individuals suffering from diabetes mellitus.[1-15] The data are interesting because reversible alterations in the serum proteins and their conjugates are related to periods of poor chemical control of diabetes and more permanent changes in the blood proteins are related to the presence of diabetic retinopathy and nephropathy. These degenerative vascular lesions histologically have been shown to contain an abundance of fat and carbohydrate stainable material.[16-19]

The blood proteins and their conjugates in vivo act as colloids and, as such, significantly influence important biophysical characteristics of the circulating blood, e.g., viscosity, osmotic pressure, and suspension stability. Changes in these physical properties in turn may affect the rate of blood flow, the caliber of small blood vessels, and the permeability through vessel walls. Therefore, modifications in the plasma protein distribution may have considerable bearing on the behavior and function of the peripheral vascular bed in general, as well as on the insidious development of degenerative changes in the smaller vessels of individuals with long-term diabetes.

Changes in the suspension stability of the blood in vivo can be evaluated from direct microscopic observation of blood flowing in the conjunctival or retinal vessels.[20-21] As the suspension stability of the blood decreases, there is a simultaneous tendency of the red cells to aggregate. In the smaller blood vessels the adhesive forces between the erythrocytes are continually influenced by the variable shearing forces present. This means that the largest aggregates usually are formed in the venules, and the smallest in the arterioles. This also means that the adhesive forces necessary to initiate aggregation must be greater in the arteriolar blood stream than those needed in the capillaries and venules, where the shear and the rate of blood flow are considerably less. Thus, a grading system of the suspension stability in vivo can be based on the location and size of the aggregates in the vascular bed.

During a microscopic study in vivo of the conjunctival blood vessels of diabetic as opposed to nondiabetic, healthy subjects, it was demonstrated that intra-

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vascular erythrocyte aggregation (I.E.A.) associated with a slowing of blood flow occurred far more frequently and extensively in the diabetics.[22] Whether this intravascular phenomenon is related to the incidence and severity of vascular lesions in the smaller blood vessels is not known. However, this might be suspected from the existence of a positive correlation between the changes in the blood proteins and the diabetic vascular disease. It was the purpose of the present study to investigate whether intravascular erythrocyte aggregation occurs more frequently in diabetics with retinopathy and nephropathy than in those without these lesions.

In diabetes, changes in the serum proteins, lipoproteins, and protein-bound carbohydrates frequently occur at the same time. Each of these protein complexes contains fractions which individually are able to induce intravascular erythrocyte aggregation. The extent to which the microcirculation is affected by each fraction of the serum proteins and their conjugates is not known. Another purpose of this study was to evaluate the influence each individual fraction of the serum proteins, lipoproteins, and protein-bound carbohydrates has upon the microcirculation and aggregation phenomenon in a large group of diabetics.

MATERIALS AND METHODS

Two different groups of subjects were used in the present investigation.

In the first group, 215 individuals were studied in order to evaluate the relationship between the incidence of pronounced intravascular erythrocyte aggregation (I.E.A.) and the presence and severity of retinopathy and nephropathy. One hundred forty-five were young diabetic patients who were with and without retinal and renal complications; the remaining 70 were asymptomatic, supposedly healthy children who were used as controls. The whole material was nearly evenly divided as to sex (106 males and 109 females). All diabetic patients received insulin which was usually a small amount of crystalline mixed with NPH 50 insulin. Of the diabetics selected, each had developed the disease before his twentieth year of age. Other than diabetes and its complications, the subjects were free from any other known disease.

After a thorough history and physical examination, all of the diabetic patients, except for a few with short duration, were examined by a qualified ophthalmologist. The cases were then separated into 3 groups: those without retinopathy, those with simple nonproliferative retinopathy, and those with retinitis proliferans. Retinitis proliferans was defined as any neovascularization.

Since diabetic glomerulosclerosis is a histologic diagnosis, the term "diabetic nephropathy" has been chosen. The presence of persistent albuminuria of at least 50 mg. per 100 ml. or more, in the urine, when not obviously due to other causes, was considered presumptive evidence of diabetic nephropathy. Furthermore, the discovery of edema and hypertension (defined as persistent elevation of the systolic blood pressure over 140 mm. Hg and diastolic blood pressure over 90 mm. Hg) provided presumptive criteria. Whenever the diagnosis was in doubt, further kidney-function tests were performed, e.g. measurement of urea clearance, phenolsulfonphthalein excretion, nonprotein nitrogen of the blood, and examination of urinary sediment.

In Table I the pertinent data are given on all subjects with regard to age at examination and, for the diabetic subjects, age at onset and duration of diabetes. It will be noted that 70 of the diabetic patients were free from vascular disease, 26 demonstrated simple retinopathy alone, 23 had proliferative retinopathy alone, and 26 exhibited a combination of retinopathy and nephropathy.

TABLE I. CLINICAL DATA ON SUBJECTS COMPRISING THE FIRST MATERIAL

GROUPS	NO. OF CASES	AGE AT ONSET OF DIABETES (YEARS ± S.D.*)	AGE AT EXAMINATION (YEARS ± S.D.)	DURATION OF DIABETES (YEARS ± S.D.)
Control patients	70	—	10.4 ± 2.6	—
Diabetics without complications	70	6.5 ± 3.1	11.0 ± 3.0	5.0 ± 3.1
Diabetics with simple retinopathy alone	26	8.0 ± 4.7	26.7 ± 7.2	18.7 ± 6.4
Diabetics with proliferative retinopathy alone	23	10.3 ± 4.9	29.1 ± 6.6	18.9 ± 6.9
Diabetics with retinopathy and nephropathy	26	11.2 ± 5.0	30.0 ± 6.7	18.8 ± 5.6

*S.D. = Standard deviation.

The examination of the conjunctival vascular bed was made independently of the clinical assessment. Therefore, the investigator was unbiased as to the result of the retinal and kidney studies. Details concerning the method for studying the conjunctival vessels have been given previously.[23-25] The degree of I.E.A., based on microscopic observations at a magnification of 48 times, was graded, with the use of the following semiquantitative criteria:

Grade 0.—Blood vessels are completely nonaggregated and homogeneous (visually and photographically demonstrable). There is rapid blood flow.

Grade 1.—Aggregates are present in the smaller venules. The flow in the larger conjunctival vessels appears homogeneous, but the flow in the small venous channels appears granulated (visually and photographically demonstrable).

Grade 2.—There is consistent aggregation in the smaller venules with intermittent aggregation in the larger ones and in the capillaries. The flow in the arterioles has a homogeneous appearance (visually and photographically demonstrable).

Grade 3.—Aggregation is present in all small blood vessels including the terminal arterioles (visually and photographically demonstrable). The aggregates may be separated by clear spaces (plasma). The linear rate of erythrocyte flow is considerably decreased in most small vessels.

The second clinical group consisted of 167 individuals and was used for the evaluation of the relationship between (1) the presence and degree of intravascular erythrocyte aggregation and (2) changes in the distribution of the serum proteins, lipoproteins, and protein-bound carbohydrates. Twenty-eight subjects were asymptomatic, nondiabetic, young hospital employees, and the remaining 139 were ambulatory diabetic patients of the Joslin Clinic. In the diabetic group selected, all had acquired diabetes before the age of 25 years. Their actual ages ranged from 14 to 41 years and they had had diabetes from 6 months to 35 years, the majority for more than 6 years. Other than diabetes, the subjects did not suffer from any condition except that some had degenerative vascular sequelae and some of the women were pregnant. All the diabetics received insulin, and all were in fairly good nutritional status. At the time of the examination, all grades of control were present except that none had severe ketoacidosis.

In all subjects, an examination of the conjunctival blood flow including an evaluation of the degree of I.E.A. was made. Immediately thereafter venous blood was drawn for the determination of total serum proteins and for paper electrophoretic fractionation of serum proteins and their conjugates. Total serum protein was determined by the biuret method.[26] Paper electrophoretic fractionation of the serum proteins was effected using a modification of the horizontal open strip method by Grassman and Hannig.[27] The electrophoretic procedure was carried out on serum applied to Schleicher and Schuell 2043A filter paper and used with a Veronal sodium-sodium acetate buffer of an ionic strength of 0.1 and a pH of 8.6. The amount of serum spotted on the filter paper was 0.005 ml. for protein, 0.01 ml. for the lipoprotein, and 0.04 ml. for the protein-bound carbohydrate determinations. The

run lasted for fifteen hours at 4° C. with 4.9 volts per centimeter of free strip.[28] The strips were stained for proteins with amido black 10 B[28], for fat with sudan black B[29], and for carbohydrate with the McManus technique (periodic acid and fuchsin sulfate).[30] The strips stained for protein were rendered translucent in a mixture of α-bromonaphthalene and white mineral oil. The electrophoretic curves for the serum components were determined on a densitometer (Photovolt Model No. 525). The relative percentages of the individual serum protein fractions were calculated from the areas measured with a compensating polar planimeter. Double determinations were made routinely on proteins, lipoproteins, and protein-bound carbohydrates.

RESULTS

Characteristics of the Microcirculation in Diabetic and Nondiabetic Subjects.—When the conjunctival vessels were observed, magnified 48 to 96 times, certain characteristics of the blood flow of asymptomatic young individuals were

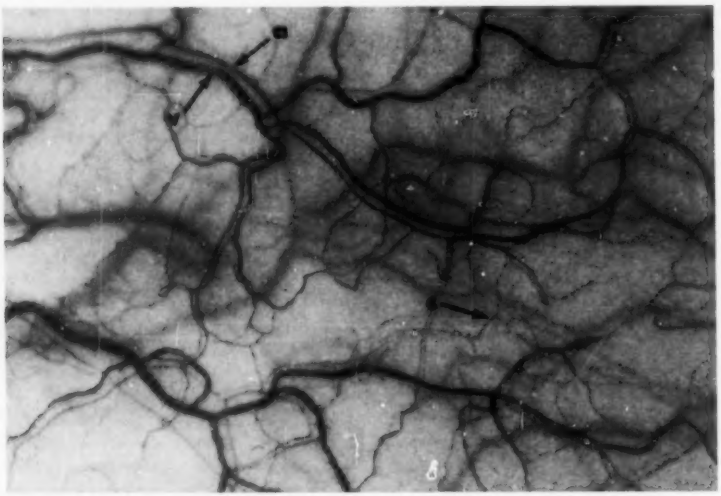

Fig. 1.—Completely unaggregated, homogeneous blood flow in branched arteriole (*a*), collecting venule (*v*), and capillary (*c*). Conjunctival vascular bed of a 14-year-old child. (×40; reduced ⅓.)

fairly similar. The rate of blood flow in the arterioles and larger venules, measuring approximately 60 to 120 μ, was so rapid that individual red cells were not distinguishable. In the smallest vessels and capillaries, and in the small venules, the blood cells circulated less rapidly, but ordinarily showed no tendency to aggregate or stick to the walls of the vessels. Of the 70 healthy children included in this study as controls, 60 showed completely nonaggregated, rapid blood flow (Fig. 1), 7 showed Grade 1, and 3 showed Grade 2 aggregation. None of the control children showed continuous retardation of blood flow with erythrocyte aggregates present in the terminal arterioles (Grade 3). This finding agrees with previous observations made on healthy individuals of more advanced age.[22]

In the diabetic subjects there was a definite tendency for the erythrocytes to clump and for the rate of flow in the smaller blood vessels to be considerably

slower. In the diabetics with severe aggregation and slow flow, aggregates were discernible in terminal arterioles, capillaries, and in all sizes of venules (Fig. 2). Within the same subject, the size of the aggregates differed depending on their location in the vascular bed. In the terminal arterioles the aggregates measured approximately 8 to 10 μ in width (the size of the diameter of an erythrocyte) and 10 to 60 μ in length. The linear rate of flow in the arterioles was definitely decreased, and at the tips of the arterioles the aggregates frequently resisted passage for a period of one-tenth second to a few seconds before being

Fig. 2.—Pronounced erythrocyte aggregation in arterioles (*a*), capillaries, and collecting venules (*v*) (Grade 3). Conjunctival vascular bed of a 32-year-old woman suffering from diabetes mellitus for 11 years and having severe diabetic retinopathy and nephropathy. (×40; reduced ⅓.)

squeezed through the capillaries. However, no permanent blocking of arterioles by aggregates was ever seen. In the venules the intravascular aggregates took the shape of irregular masses of red cells and grew much larger in size varying from approximately 10 to 60 μ in width and from 10 to 200 μ in length. Thus from their microscopic shapes and sizes alone, it would appear impossible that the aggregates in the venous circulation consisted of simple rouleaux, as might be the case for the aggregates formed in the arterioles. The size of the aggregates in the venules appeared, in part, to depend upon the rate of flow, i.e.,

TABLE II. RELATIONSHIP BETWEEN IN VIVO ERYTHROCYTE AGGREGATION AND VASCULAR PATTERN-CHANGES IN DIABETICS

GROUP	NORMAL VASCULAR PATTERN		VASCULAR PATTERN-CHANGE I		VASCULAR PATTERN-CHANGE II		TOTAL
	NO. OF SUBJECTS	NO. OF SUBJECTS WITH SEVERE AGGREGATION	NO. OF SUBJECTS	NO. OF SUBJECTS WITH SEVERE AGGREGATION	NO. OF SUBJECTS	NO. OF SUBJECTS WITH SEVERE AGGREGATION	
Diabetics without complications	35	3	33	9	2	2	70
Diabetics with complications:							
Diabetics with simple retinopathy	8		17		1		26
Diabetics with proliferative retinopathy	6 } 15	7	14 } 46	30	3 } 14	14	23
Diabetics with retinopathy and nephropathy	1		15		10		26
Per cent cases with severe aggregation	50	10	79	39	16	16	145
	20%		49%		100%		

the amount of shearing force present, and the caliber of the vessels. Large, heavy aggregates in static venous channels tended to sediment to the lower side of the vessels (Fig. 3). Occasionally in such cases a tight clump of red cells was observed to be obstructing a venule for a period of days before the clump disappeared and allowed for the microcirculation to continue. The color of the clump might change from red to dark purple; however, from the microscopic study it was impossible to determine whether this was a true microthrombus or not. It was found that the degree of aggregation varied widely from one diabetic individual to another depending on factors such as control of diabetes, accompanying infections, and the extent and degree of vascular complications.

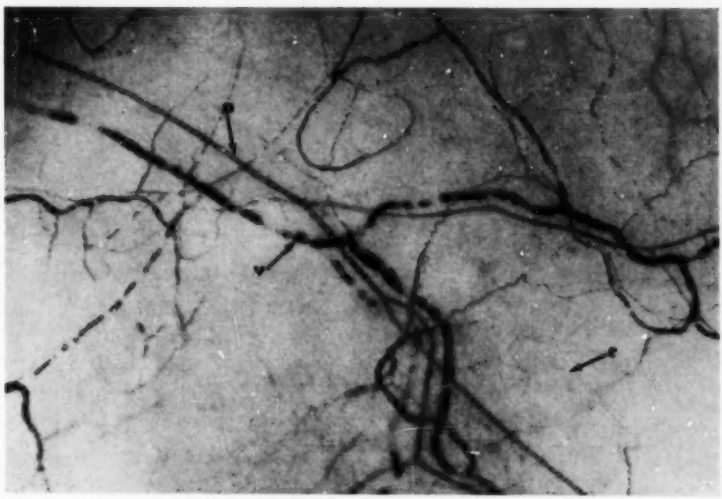

Fig. 3.—Pronounced erythrocyte aggregation in collecting venules, terminal arterioles and capillaries (Grade 3) in a 30-year-old man suffering from diabetes mellitus for 19 years and having severe diabetic retinopathy and nephropathy with no hypertension. Branched arteriole (*a*), capillary (*c*), and collecting venule (*v*). (×40; reduced ⅛.)

Relationship of Generalized Erythrocyte Aggregation In Vivo to the Presence and Severity of Retinopathy and Nephropathy.—Because examination of the supposedly healthy children demonstrated that the presence of aggregates exclusively in the venules (Grades 1 and 2) was not inconsistent with apparent health and well-being, only continuous retardation of the erythrocyte flow with aggregates in the arterioles (Grade 3) has been considered significantly abnormal. Fig. 4 shows the result of the analysis relating the incidence of pronounced aggregation (Grade 3) to the presence and severity of vascular disease. It will be noted that with an increasing degree of vascular complications there is an increasing tendency toward the occurrence of generalized erythrocyte aggregation in vivo associated with slowing blood flow. A chi-square test shows that this relationship is highly significant ($X^2 = 47.9$; $n = 3$; $p < 0.01$). Among the young diabetic patients, pronounced aggregation was encountered in 14 of

the 70 without complications (20 per cent), in 10 of the 26 with simple retinopathy (38 per cent), in 19 of the 23 with proliferative retinopathy (83 per cent), and in 22 of the 26 with retinopathy and nephropathy (85 per cent). Even though the percentage difference between the incidence of erythrocyte aggregation of diabetics with proliferative retinopathy alone and diabetics with retinopathy and nephropathy is insignificant, the diabetics with nephropathy showed more pronounced Grade 3 aggregation than those without nephropathy. The high

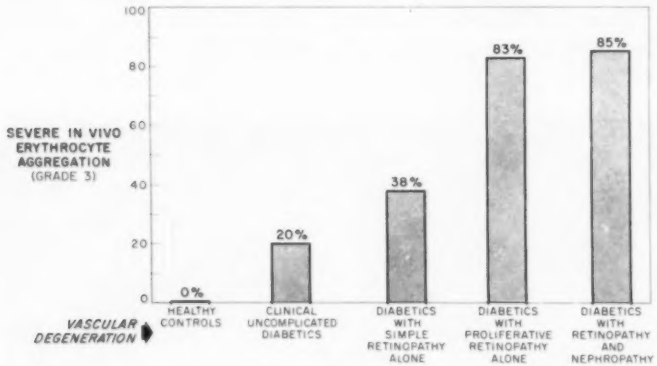

Fig. 4.—Relationship between erythrocyte aggregation in vivo and retinopathy and nephropathy.

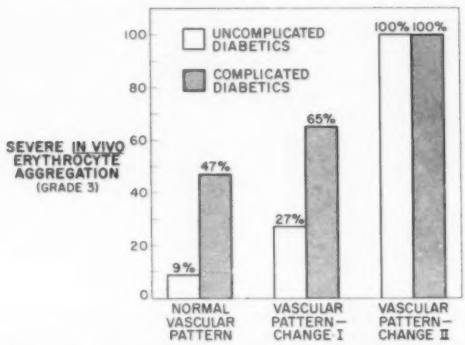

Fig. 5.—Relationship between erythrocyte aggregation in vivo and vascular pattern-changes in complicated and uncomplicated diabetics.

incidence of intravascular erythrocyte aggregation among the patients with proliferative retinopathy is probably explained by the finding that some of these patients exhibited slight albuminuria, i.e., less than 50 mg. per 100 milliliters. These diabetics, therefore, might have slight kidney damage but according to the definition would not be included among the patients with nephropathy.

Relationship of Generalized Erythrocyte Aggregation In Vivo to Caliber Changes in the Arterioles and Venules.—In a previous study it was demonstrated that young diabetic patients show various degrees of reversible caliber changes in the arterioles and venules which in some patients correlate with the degree

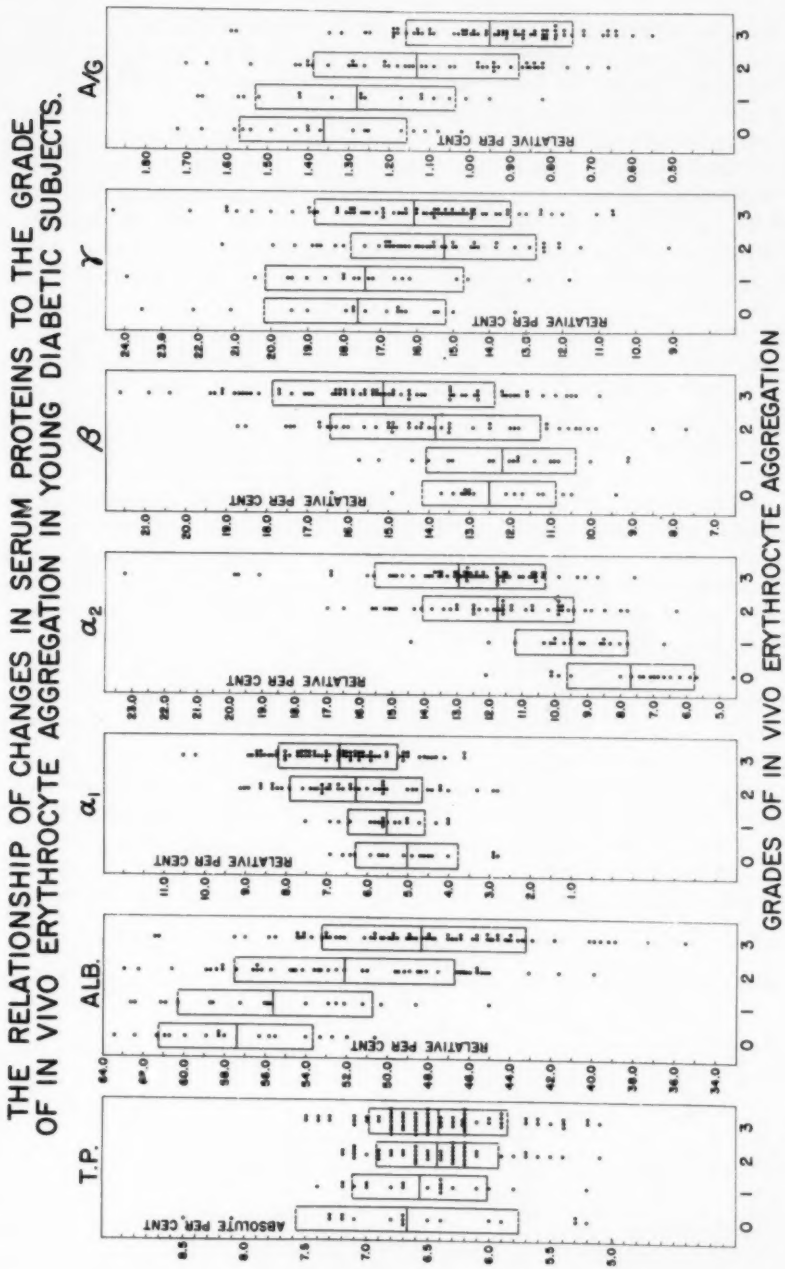
THE RELATIONSHIP OF CHANGES IN SERUM PROTEINS TO THE GRADE OF IN VIVO ERYTHROCYTE AGGREGATION IN YOUNG DIABETIC SUBJECTS.
T.P.
ABSOLUTE PER CENT
ALB.
RELATIVE PER CENT
α_1
RELATIVE PER CENT
α_2
RELATIVE PER CENT
β
RELATIVE PER CENT
γ
RELATIVE PER CENT
A/G
RELATIVE PER CENT
0 1 2 3
GRADES OF IN VIVO ERYTHROCYTE AGGREGATION

Fig. 6.

TABLE III*A*. RELATIONSHIP OF INTRAVASCULAR ERYTHROCYTE AGGREGATION (I.E.A.) TO SERUM PROTEIN COMPOSITION IN HUMAN SUBJECTS

GROUP	NUMBER OF DETERMINATIONS	TOTAL PROTEIN GRAM PER CENT	ALBUMIN PER GLOBULIN RATIO	PROTEIN FRACTIONS				
					GLOBULINS			
				ALBUMIN PER CENT ± S.D.	α_1 PER CENT ± S.D.	α_2 PER CENT ± S.D.	β PER CENT ± S.D.	γ PER CENT ± S.D.
				(COMPOUNDS EXPRESSED AS PERCENTAGES OF TOTAL PLANIMETER AREAS)				
Individuals without I.E.A.	18	6.7 ± 0.91	1.36 ± 0.208	57.4 ± 3.78	5.0 ± 1.27	7.7 ± 1.91	12.5 ± 1.64	17.6 ± 2.58
Individuals with grade 1 I.E.A.	19	6.6 ± 0.55	1.28 ± 0.247	55.6 ± 4.90	5.5 ± 0.93	9.5 ± 1.70	12.2 ± 1.83	17.4 ± 2.72
Individuals with grade 2 I.E.A.	48	6.4 ± 0.50	1.13 ± 0.253	52.1 ± 5.43	6.3 ± 1.64	11.7 ± 2.33	13.8 ± 2.60	15.2 ± 2.53
Individuals with grade 3 I.E.A.	72	6.4 ± 0.57	0.95 ± 0.207	48.3 ± 5.11	6.7 ± 1.47	12.9 ± 2.65	15.1 ± 2.74	16.1 ± 2.68

of metabolic disturbance.[32, 33] The pathologic vascular responses deviating from the normal vascular pattern were designated "vascular pattern-change I and II." The normal vascular pattern was characterized by an arteriolar-venular diameter ratio (A-V ratio 1:3 to 1:2). Vascular pattern-change I was characterized by a general dilatation of the collecting venules and an occasional narrowing of the arterioles. Consequently the A-V ratio was variably decreased from 1:4 to 1:8. Vascular pattern-change II was characterized by a general constriction of all vascular elements and the A-V ratio tended to increase to 1:1. It was further demonstrated that the pattern changes in the small blood vessels of diabetic patients were correlated with the presence of retinopathy and nephropathy.[32]

The present data demonstrate a correlation between the incidence of erythrocyte aggregation in vivo and the presence of retinopathy and nephropathy. Therefore, this might indicate a relationship between the intravascular erythrocyte aggregation and the reversible changes in the small blood vessels. Table II correlates the incidence of severe aggregation to the vascular patterns observed in the 145 diabetic subjects. Fifty exhibited normal vascular pattern, 79 showed vascular pattern-change I, and 16 showed vascular pattern-change II. It is seen that diabetics exhibiting vascular pattern-change I have Grade 3 aggregation more frequently (49 per cent) than those showing normal vascular pattern (20 per cent) but less frequently than those demonstrating vascular pattern-change II (100 per cent). Fig. 5 shows that the relationship between the presence of pronounced aggregation and the vascular patterns also remains significant when the subjects in each group are divided into uncomplicated and complicated cases. The complicated cases generally showed more pronounced aggregation than the corresponding uncomplicated group.

Relationship of Changes in the Serum Proteins, Lipoproteins, and Protein-Bound Carbohydrates to the Degree of Intravascular Erythrocyte Aggregation.— In Table III*A* and in Fig. 6, the relationship between the degree of intravascular erythrocyte aggregation and the serum protein composition, based on a total of 157 determinations, is presented. The distribution of the degree of I.E.A. in this selected group of diabetics and a few nondiabetics was as follows. Eighteen

TABLE III*B*. STATISTICAL ANALYSIS OF THE DATA ON SERUM PROTEINS FROM CONTROL AND I.E.A. GROUPS

GROUPS COMPARED	TOTAL PROTEIN	ALBUMIN PER GLOBULIN RATIO	ALBUMIN	GLOBULINS			
				α_1	α_2	β	γ
Control-1	N.S.*	N.S.	N.S.	N.S.	$p < 0.01$ CR = 3.0	N.S.	N.S.
1-2	N.S.	N.S.	$p < 0.01$ CR = 2.6	N.S.	$p < 0.01$ CR = 4.3	$p < 0.01$ CR = 2.8	$p < 0.01$ CR = 3.0
2-3	N.S.	$p < 0.01$ CR = 4.0	$p < 0.01$ CR = 3.8	N.S.	$p < 0.01$ CR = 2.6	$p < 0.01$ CR = 2.6	N.S.
Control-2	N.S.	$p < 0.01$ CR = 3.7	$p < 0.01$ CR = 4.5	$p < 0.01$ CR = 3.3	$p < 0.01$ CR = 7.2	N.S.	$p < 0.01$ CR = 3.3
Control-3	N.S.	$p < 0.01$ CR = 7.5	$p < 0.01$ CR = 8.5	$p < 0.01$ CR = 4.9	$p < 0.01$ CR = 9.5	$p < 0.01$ CR = 5.2	N.S.

*N.S. equals: not significant.

TABLE IV*A*. RELATIONSHIP OF INTRAVASCULAR ERYTHROCYTE AGGREGATION (I.E.A.) TO SERUM LIPOPROTEIN COMPOSITION IN HUMAN SUBJECTS

GROUP	NUMBER OF DETERMINATIONS	TOTAL AREA	α AREA ± S.D.	β AREA ± S.D.
Individuals without I.E.A.	24	10.2 ± 3.98	2.0 ± 0.69	8.2 ± 3.62
Diabetics with grade 2 I.E.A.	44	12.5 ± 3.70	2.8 ± 1.20	9.7 ± 3.08
Diabetics with grade 3 I.E.A.	60	14.1 ± 3.49	3.1 ± 0.99	11.0 ± 2.93

individuals (11 per cent) showed completely nonaggregated, homogeneous, rapid blood flow, 19 (12 per cent) had Grade 1, 48 (31 per cent) had Grade 2, and 72 (46 per cent) showed pronounced aggregation (Grade 3). Statistical analysis* of the data is given in Table III*B*. It will be noted that with a progressive decrease in the albumin-globulin ratio from an average of 1.36 to 0.95, there is a simultaneous tendency for more pronounced aggregation to occur ($p < 0.01$). This change in the albumin-globulin ratio is produced by a decrease in the albumin and a simultaneous increase in the globulin components. The albumin fraction decreased from 57.4 per cent in the subjects having no aggregation to 48.3 per cent in those showing Grade 3 I.E.A. ($p < 0.01$). Simultaneously, significant increases occurred in the α_1-, α_2-, and β-globulins. The changes in the α_2-globulin fraction correlated most significantly with the degree of I.E.A. The average α_2-globulin was 7.7 per cent in the individuals without I.E.A. and increased to 12.9 per cent in the subjects with pronounced aggregation ($p < 0.01$). Similarly the β-globulin fraction increased progressively from 12.5 per cent to 15.1 per cent in comparing the individuals without I.E.A. to those with increasingly severe I.E.A. ($p < 0.01$). A less striking, although significant, increase occurred in the α_1-globulin fraction from the control group to the group showing Grade 3 aggregation, 5.0 per cent to 6.7 per cent. There were no significant changes in the total protein in any of the groups. Therefore the albumin, α_1-, α_2-, and β-globulins can be expressed also in gram per cent. No specific relationship was found between any changes in the γ-globulin and the degree of aggregation in this particular group.

In Table IV*A* the relationship between the serum lipoprotein composition and the degree of I.E.A., based on a total of 128 determinations is given. It is important to emphasize that in this series the 24 individuals without I.E.A. were all nondiabetic and supposedly healthy, while the subjects in groups 2 and 3 consisted only of diabetic patients. Statistical analysis of the data is given in

TABLE IV*B*. STATISTICAL ANALYSIS OF THE DATA ON SERUM LIPOPROTEINS FROM CONTROL AND I.E.A. GROUPS

GROUPS COMPARED	TOTAL AREA	α	β
Control-2	N.S.	CR = 3.5	N.S.
Control-3	CR = 4.2	CR = 5.7	CR = 3.4
2-3	N.S. (CR = 2.2)	N.S. (CR = 1.3)	N.S. (CR = 2.1)

*The probability, $p < 0.01$, is calculated from the *critical ratio* (CR), which must be greater than 2.6 for the assumption to be made that the 2 means are actually different. $CR = \frac{\Delta_m}{\sigma_m}$, where Δ_m equals the difference between the 2 means and σ_m equals the standard error of the difference between the 2 means.

TABLE V*A*. RELATIONSHIP OF INTRAVASCULAR ERYTHROCYTE AGGREGATION (I.E.A.) TO SERUM PROTEIN-BOUND CARBOHYDRATES IN HUMAN SUBJECTS

GROUP	NUMBER OF DETERMINATIONS	TOTAL AREA	ALBUMIN AREA ± S.D.	α_1 AREA ± S.D.	α_2 AREA ± S.D.	β AREA ± S.D.	γ AREA ± S.D.
Individuals without I.E.A.	24	8.2 ± 3.12	1.1 ± 0.64	1.7 ± 0.67	1.9 ± 0.72	1.9 ± 0.82	1.6 ± 0.58
Diabetics with grade 2 I.E.A.	37	9.7 ± 1.84	1.0 ± 0.33	1.9 ± 0.44	2.8 ± 0.63	2.4 ± 0.63	1.6 ± 0.51
Diabetics with grade 3 I.E.A.	57	9.5 ± 1.54	0.8 ± 0.31	1.9 ± 0.31	2.8 ± 0.58	2.3 ± 0.55	1.6 ± 0.46

TABLE V*B*. STATISTICAL ANALYSIS OF THE DATA ON SERUM PROTEIN-BOUND CARBOHYDRATES FROM CONTROL AND I.E.A. GROUPS

GROUPS COMPARED	TOTAL AREA	ALBUMIN	α_1	α_2	β	γ
Controls-2	N.S.	N.S.	N.S.	CR = 5.0	N.S.	N.S.
Controls-3	N.S.	N.S. (CR = 2.2)	N.S.	CR = 5.7	N.S.	N.S.
2-3	N.S.	CR = 3.4	N.S.	N.S.	N.S.	N.S.

Table IV*B*. It will be noted that with a progressive increase in both the total area of lipoprotein and in the two fractions, α- and β-lipoproteins, there is an increasing tendency for aggregation to occur ($p < 0.01$). The increases in total lipoproteins and their fractions were not quite significant when the diabetics with Grade 2 were compared with the diabetics with Grade 3 I.E.A. This might indicate that the increases in lipoproteins are more closely related to the presence of diabetes than to increasing degrees of I.E.A.

In Table *VA* the relationship between the serum protein-bound carbohydrates and the degree of I.E.A., based on a total of 118 determinations, is given. Again the 24 subjects without intravascular erythrocyte aggregation were nondiabetic and healthy, while the subjects in groups 2 and 3 consisted only of diabetics. Statistical analysis of the data is given in Table V*B*. In comparing the single fractions of protein-bound carbohydrates to increasing severity of aggregation, the only significant change was an increase in the α_2 fraction from 1.9 to 2.8 area units. However, it will be noted that there is no change in this α_2 fraction from the diabetics with Grade 2 I.E.A. to those with Grade 3 I.E.A. This indicates that the increase in protein-bound carbohydrate reflects the presence of diabetes rather than a true relationship to the degree of aggregation.

DISCUSSION

The data presented relate biochemical changes in the blood (proteins and their conjugates) to biophysical alterations in the smaller blood vessels. In diabetic subjects, changes occur in the composition of the serum proteins, lipoproteins, and protein-bound carbohydrates. The relative amount of serum albumin decreases and simultaneously the proportions of α_1-, α_2-, and β-globulins increase. The α- and β- lipoproteins as well as the α_2 fraction of the serum protein-bound carbohydrates also increase. As these protein changes occur in

vivo and become progressively more marked, the tendency of the circulating erythrocytes to aggregate becomes greater. This phenomenon appears to be related more closely to an increase in the α_2-globulin and a decrease in the albumin than to an increase in the β-globulin, α- and β-lipoproteins, and α_2 protein-bound carbohydrates. Therefore, the degree of aggregation is influenced more by changes in the serum proteins than by the alterations in the lipoproteins or protein-bound carbohydrates. The erythrocyte aggregates, as observed in the conjunctival vessels, may intermittently resist passage through the tips of the arterioles and in the venules larger aggregates may sediment to the lower side of the vessels. The rate of flow, particularly in the capillaries and in the venules, becomes abnormally slow and thus provides a condition which favors microthrombus formation and deposition of carbohydrate-lipid-protein complexes in the walls of the small blood vessels.

The presence of such a relationship between changes in the protein distribution and the degree of erythrocyte aggregation in vivo is not surprising in view of the extensive studies relating the in vitro sedimentation rate to increased concentrations of fibrinogen and globulins.[34, 35] Since the erythrocyte sedimentation rate is a measure of the tendency of red cells to form rouleaux, the results, therefore, indirectly support the contention that the erythrocyte aggregation in vivo is related to rouleaux formation and opposes the view expressed by Knisely and co-workers[36] that intravascular erythrocyte aggregation, termed "sludged blood," is an entirely different phenomenon.

With increasing degree of retinopathy and nephropathy there is an increasing tendency for extensive intravascular erythrocyte aggregation to occur. This relationship would be expected since the presence of retinopathy and/or nephropathy is associated with changes in the distribution of serum proteins, lipoproteins, and protein-bound carbohydrates. The relationship between the vascular degeneration and the high incidence of aggregation is thus a reflection of the changes occurring in the blood proteins and their conjugates. It is of interest to note that 20 per cent of the young diabetic patients showing no evidence of vascular disease had pronounced intravascular erythrocyte aggregation. This supports the view that changes in the blood proteins may precede the degenerative alterations in the vessels.[15]

It has been reported previously that abnormal vascular responses occur in the conjunctival vascular bed of patients with diabetes.[31] These responses were correlated to the incidence of retinopathy and nephropathy.[32] In the present study, a relationship is found between the incidence of pronounced aggregation, i.e., the disturbance in the plasma proteins, and the presence of vascular pattern-changes I and II. This relationship is of considerable interest. Bloch,[37-39] in studying the conjunctival vascular bed in patients suffering from poliomyelitis and myocardial infarction, described a similar sequence of vascular responses in the arterioles and venules during the acute phase of these diseases to that observed in poorly controlled young diabetics. Because Bloch observed intravascular erythrocyte aggregation simultaneously with the caliber changes in the blood vessels, he believed that the clumping phenomenon caused the venular

dilatation (vascular pattern-change I). The data obtained in this study demonstrate no cause and effect relationship between the aggregation phenomenon and the venular dilatation in diabetes. This is clearly evident since, of the diabetic individuals with complications who have normal vascular pattern, as many as 47 per cent showed pronounced aggregation (Fig. 5). Therefore, the correlation between the aggregation and the vascular pattern changes suggests that both the aggregation, i.e., the protein changes, as well as the vascular pattern changes, are manifestations of tissue responses to acute alterations in the metabolism of the diabetic subjects.

SUMMARY

1. In order to evaluate the relationship between the incidence of intravascular erythrocyte aggregation ("sludged blood") and the presence and severity of retinopathy and nephropathy, direct biomicroscopic observations of the microcirculation in the conjunctival vascular bed were made in 70 asymptomatic, supposedly healthy children and 145 young diabetics, with and without retinal and renal complications. It is found that intravascular erythrocyte aggregation occurs far more frequently and more extensively in the diabetics than in the nondiabetic controls. With increasing degrees of diabetic vascular complications there is an increasing tendency for generalized erythrocyte aggregation in vivo with slowing of blood flow in the small blood vessels to occur.

2. Comparative studies were made relating the degree of intravascular erythrocyte aggregation to the composition of the serum proteins, lipoproteins, and protein-bound carbohydrates as determined by paper electrophoresis. In 157 determinations, a significant relationship was shown between intravascular erythrocyte aggregation (I.E.A.) and an increase in α_1-, α_2-, and β-globulins and a simultaneous decrease in the albumin fraction of serum. One hundred twenty-eight serum lipoprotein determinations show a correlation between the degree of aggregation and the amount of total lipoprotein and its two component fractions, α- and β-lipoproteins. One hundred eighteen serum protein-bound carbohydrate determinations show no direct relationship between their component fractions and the degree of aggregation.

3. A correlation is present between the incidence of intravascular erythrocyte aggregation and the presence of vascular pattern-changes I and II in the conjunctival vascular bed. This correlation suggests that both the aggregation phenomenon, i.e., the protein changes, as well as the vascular pattern changes, are independent manifestations of tissue responses to acute alterations in the metabolism of the diabetic subjects.

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COBALT AS A PRODUCER OF PORPHYRINURIA AND POLYCYTHEMIA

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COBALT is known as a powerful accelerator of erythropoiesis. A marked polycythemia can be produced by cobalt in many species of animals and in several situations where other erythropoietic factors are without any effect. Anemia in the rat, produced by a diet low in protein, can be reversed to polycythemia by the administration of cobalt.[1] Similarly, cobalt not only prevents anemia following hypophysectomy in the rat, but it actually induces a marked elevation in the number of erythrocytes and in hemoglobin from the preoperative level.[2] Adrenalectomized rats that are treated with cobalt develop anemia 20 days after operation, but during the period of 30 to 65 days following adrenalectomy, a significant elevation of blood values occurs which is explained by the development of accessory adrenal cortical tissue.[3]

Cobalt frequently exerts a pronounced effect in human renal anemia[4] and the anemia of prematurity, although, considering the severe side effects observed in the course of cobalt therapy, the indication must be critically established. Side effects, associated with the cobalt therapy, are, according to Reimold,[5] disturbance of growth, lack of appetite and vomiting, claudication and angina pectoris, occasional inhibition of erythropoiesis, inhibition of growth of tumors, and development of goiter. Cobalt induced goiter with cardiomegaly and congestive failure in a newborn infant has been reported by Little and Sunico.[6] The goitrogenic action of cobalt has also been demonstrated by Antila and associates.[7] Fisher's other observation,[3] that in rats treated with cobalt the ratio of adrenals to body weight is significantly higher than in control animals, supports the theory that the endocrine system is involved in the induction of polycythemia by cobalt. That the spleen is also affected by cobalt has been shown by Hopps and co-workers[8] who demonstrated histologic changes in the structure of the spleen as a result of cobalt chloride injection.

The mechanism of cobalt polycythemia has been studied in many animal experiments. Orten and Bucciero[9] observed that supplementation of the cobalt-containing diet with cysteine inhibits the production of polycythemia in rats and that histidine acts similarly but is less effective. The authors suggested that cobalt may produce polycythemia by binding sulfhydryl (SH-) or perhaps other groups active in cellular respiration thus leading to a simulated cellular anoxia and, in turn, to compensatory polycythemia. In this connection the studies of Levy and associates[10] are worthy of consideration. They tested the

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TABLE I. SUBSTANCES TESTED FOR THEIR ABILITY TO PREVENT OR REDUCE THE POLYCYTHEMIC EFFECT OF COBALT

SUBSTANCE	TEST ANIMAL	MODE OF ADMINISTRATION	ABLE TO COUNTERACT	AUTHOR
Ascorbic acid	Rabbit	Intravenously	Yes	Barron and Barron[11] Levey[12]
	Rat	Intravenously Orally	No	Levey[12]
Isoascorbic acid	Rabbit	Intravenously	Yes	Levey[12]
	Rat	Intravenously Orally	No	Levey[12]
Gluconascorbic acid	Rat	Intravenously Orally	No	Levey[12]
ETDA	Rabbit	Intravenously		
Choline	Rat	Subcutaneously	Yes	Post[13]
	Dog	Orally	Yes	Davis[14]
	Rat	Orally	No	Bucciero and Orten[15]
Cysteine	Rat	Orally	Yes	Orten and Bucciero[9]
Cytochrome C	Rat		No	Levey[16]
Histidine	Rat	Orally	Yes	Orten and Bucciero[9]
Methionine	Rat	Orally	No	Orten and Bucciero[9]
Methylene blue	Rabbit	Intraperitoneally	Yes	Wesley[17]
Nicotinamide	Rabbit		Yes	Gobell[18]

effect of cobalt in concentration of 10^{-3} M on the activity of 10 different enzyme systems in tissue homogenates. Under aerobic conditions cobalt had a marked inhibitory action on succinoxidase and choline oxidase, which are both -SH dependent, and on 2 -SH-independent enzyme systems: cytochrome oxidase and catalase. Choline dehydrogenase and succinic dehydrogenase were inhibited anaerobically by cobalt, although the latter enzyme was much less sensitive under these conditions than under aerobic conditions.

Besides cysteine and histidine, many other substances have been tested for their ability to prevent or reduce the polycythemic effect of cobalt. These substances are summarized in Table I.

Barron and Barron[11] suggested that cobalt polycythemia may be due to inhibition by cobalt of the respiratory function of immature red cells. This theory could not be confirmed by Warren and co-workers[19] with in vitro experiments. Bucciero and Orten[20] did not find any evidence of a decrease in the oxygen capacity of the blood of the cobalt-treated polycythemic rats, nor did they observe any methemoglobinemia. The effect of cobalt on heme synthesis by bone marrow in vitro has been studied by Laforet and Thomas[21] who observed that cobalt at concentration greater than 10^{-4} M inhibits heme synthesis, but does not interfere with oxygen consumption until a concentration of 10^{-2} M is reached. They concluded that cobalt does not produce polycythemia by mechanism of bone marrow anoxia. The observation of Wesley[22] that the oxygen consumption of rats after an intraperitoneal injection of cobalt is remarkably diminished indicates, however, that cobalt interferes with some respiratory functions of the organism.

The side effects, reported to occur during cobalt therapy, and the animal experiments mentioned above both point to intoxication. Since porphyrinuria is a frequent finding in heavy metal intoxications, the urinary porphyrine excretion of rats treated with cobalt was analyzed in the present study.

MATERIAL AND METHODS

Thirty adult female albino rats of Wistar strain were used in the experiments. The animals, weighing from 170 to 200 grams were divided into 3 groups of 16 rats each.

In Group I, 4 mg. of cobalt chloride ($CoCl_2 \cdot 6H_2O$)* was administered to each rat subcutaneously, in aqueous solution, every day for 4 weeks; this corresponds to 1 mg. of cobalt daily. Porphyrin and hemoglobin determinations were made from these rats before and after the first, fourth, eighth, fourteenth, and twenty-eighth injections of cobalt. All animals were weighed before and after the experimental period of 4 weeks which ended when the animals were killed. The adrenals were then weighed and routine histologic specimens were prepared from the liver, kidney, spleen and adrenal; these were stained with hematoxylin-eosin. Also, bone marrow smears were made and stained with the May-Grünwald-Giemsa method.

The rats in Group II received only 0.25 mg. of cobalt daily. In every other respect this group was handled like Group I.

The remaining 10 rats formed a control group.

Determination of Urinary Porphyrins.—Because the volume of urine excreted by a single rat in 24 hours is too small for porphyrin determinations by the method used, the urine excreted in 24 hours by all the rats together in each group was collected in the same bottle, and the total sample was analyzed. The result was then divided by the number of rats (10), so that the average amount of porphyrin excreted by a single rat in 24 hours was obtained. Both ether-soluble coproporphyrin and ether-insoluble uroporphyrin were determined.

Coproporphyrin.—Extractions were made by the method presented by Koskelo,[23] which is a modification of the method of Schwartz and associates.[24] To 10 ml. of urine was added 10 ml. of buffered acetic acid solution (4 volumes of glacial acetic acid plus 1 volume of saturated aqueous sodium acetate solution). The resulting acid urine was extracted with 75 ml. of peroxide-free ether, using in all cases an identical extraction time of 15 minutes. The ether layer was washed twice with 20 ml. portions of 1 per cent sodium acetate. The coproporphyrin chromogen was converted to coproporphyrin by washing the ether once with 0.005 per cent aqueous iodine. Finally coproporphyrin was extracted from ether with 5 ml. portions of 1.5 N hydrochloric acid until no red fluorescence could be observed in the hydrochloric acidphase when examined in ultraviolet light.

The concentration of coproporphyrin was determined spectrophotometrically from the acid solution, using a Beckman spectrophotometer. The true extinction of coproporphyrin was calculated according to the following correction formula presented by With[25]:

$$d^{corr} = \frac{2E_{max} - (E_{430} + E_{350})}{1.835}$$

where E is measured extinction, max is Soret maximum (401 to 402 mμ), and d^{corr} is extinction due to coproporphyrin alone.

The extinction values are transformed to micrograms per 1 ml. of solution in the photometric cell by means of the extinction coefficient for pure preparations of porphyrin. The value of $E\ {}^{1\%}_{1\ cm.}$ (calculated extinction for a solution containing 1 Gm. per 100 ml. and read in a 1 cm. layer) is 666F for coproporphyrin.[25] Then value of $E\ {}^{1\%}_{1\ cm.} = 666F$ corresponds to the value of $d\ {}^{corr}_{1\ cm.} = 0.6667$ for a solution containing 1 mcg. per 1 ml. (10^{-4} Gm. per 100 ml.). The average amount of coproporphyrin excreted in urine in 24 hours by a rat can then be calculated according to the following formula:

$$\text{Coproporphyrin in micrograms} = \frac{[2E_{max} - (E_{350} + E_{430})]\ a \times b}{1.835 \times 0.6667 \times 10 \times N}$$

where a is the volume of hydrochloric acid (in ml.) used in extraction, b is the volume of urine (in ml.) excreted in 24 hours by the rats in a group, and N is number of the rats (10) in the group.

*Pro analysi, Merck.

Uroporphyrin.—After coproporphyrin is extracted with ether, the urine is made acid (pH 1.5) with the aid of concentrated hydrochloric acid. Uroporphyrin can then be extracted with cyclohexanone. Extraction was made twice with 50 ml. of cyclohexanone and the extraction time was 10 minutes. To the cyclohexanone layer was added a similar amount (100 ml.) of petroleum ether. Then uroporphyrin was extracted from this mixture with 5 ml. portions of 1.5 N hydrochloric acid until no fluorescence could be seen in ultraviolet light. The concentration of uroporphyrin was determined spectrophotometrically like the concentration of coproporphyrin. The Soret maximum of uroporphyrin in 1.5 N hydrochloric acid is 404 to 405 mμ, and the background is read at 380 and 430 mμ. The correction divisor for uroporphyrin is 1.844 and the value of $E\ {}^{1\%}_{1\ cm.} = 6{,}535.^{25}$

Hemoglobin.—Blood, obtained from the tail veins for hemoglobin determination, was analyzed by routine method in the spectrophotometer. The average value of hemoglobin in each group was then expressed in grams per 100 ml. of blood.

RESULTS

The rats in the first group already looked ill after the third injection. They were depressed and slow. When the cobalt treatment was continued, the symptoms became more and more apparent. The weight of the rats decreased approximately 11 per cent during the experiment. On the other hand, the adrenals increased remarkably in size: the average adrenal to body weight ratio was $72:1.0 \times 10^5$, whereas this value was $39:1.0 \times 10^5$ in the control animals.

The impairment of the general condition was less pronounced in the rats of the second group, but the decrease in weight (8 per cent) was of approximately the same magnitude as was the decrease in the rats treated with 1 mg. of cobalt. The adrenal to body weight ratio in the second group was $54:1.0 \times 10^5$, which is also significantly higher than the control ratio.

The average urinary coproporphyrin excretion by a single rat in 24 hours before and after the cobalt treatment in Groups I and II is represented diagrammatically in Figs. 1 and 2, respectively. In addition to the black columns, which represent the amount of coproporphyrin in micrograms, the white ones are inserted to show the changes in hemoglobin. The number of injections that the animals had received before the determinations were made are indicated below the columns.

The amount of coproporphyrin, excreted by 1 rat seemed to be quite constant before cobalt treatment. Several determinations were made, and the values obtained varied between 0.308 and 0.364 μg, the mean value being 0.340 μg. The results of analyses made side by side from the same sample did not deviate more than 5 per cent from each other.

In Group I, where the daily dose of cobalt was 1 mg., the amount of coproporphyrin in urine was increased significantly even after the first injection. After several injections this increase became more and more apparent and after the twenty-eighth injection, the excretion of coproporphyrin was more than doubled. The amount of hemoglobin decreased at first. The lowest value, obtained after the eighth injection, was 1.2 Gm. below the original level. After the fourteenth injection, however, this level was exceeded and polycythemia appeared. The increase was about 20 per cent after 28 injections.

No signs of increased coproporphyrin excretion could be observed in the rats receiving 0.25 mg. of cobalt only. The values obtained from this group remained within control limits the whole time. In spite of this, the hemoglobin values behaved as in the first group: at first a slight lowering was observed; this was then followed by a polycythemic reaction, and the last value determined was also about 20 per cent higher than the control level.

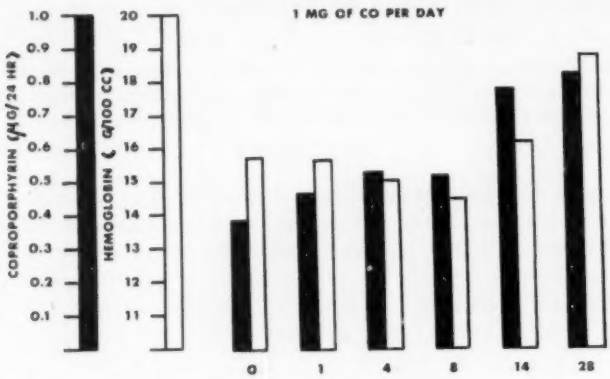

Fig. 1.—The average urinary excretion of coproporphyrin by a rat administered 1 mg. of cobalt daily. The determinations were made before and after the first, fourth, eighth, fourteenth, and twenty-eighth injections.

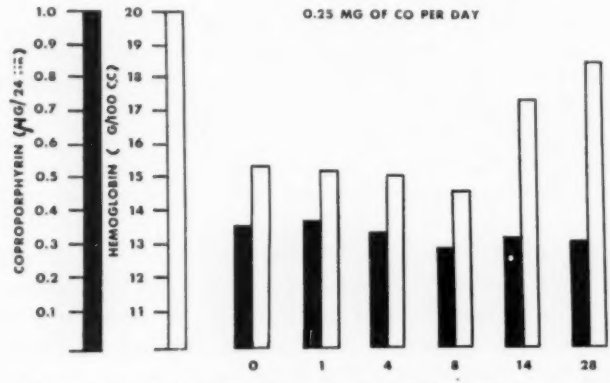

Fig. 2.—The average urinary excretion of coproporphyrin by a rat administered 0.25 mg. of cobalt daily. The determinations were made before and after the first, fourth, eighth, fourteenth, and twenty-eighth injections.

The amount of uroporphyrin proved to be so minimal in all experiments that no exact calculations could be obtained.

Findings, made by routine histopathologic examination at the end of the cobalt treatment were rather scanty and nonspecific. In Group I where the animals were treated with 1 mg. of cobalt daily, changes of the spleen were constantly observed. There was disintegration of the follicular structure and lessening of the amount of lymphoid tissue, in contrast to hyperplasia of the

reticular cells. The same phenomenon was seen clearly in the 0.25 mg. group as well, although it was less marked. In both groups the adrenals showed hypertrophy and hyperemia of the cortical layer. In Group I, the liver of some animals showed cloudy swelling and edema in periportal areas, but this was not a constant finding. No obvious changes could be observed in the kidneys, obtained from the animals treated with cobalt. In bone marrow smears, the number of erythroblasts was apparently increased in contrast to the normal bone marrow of the rat which is normally rather poor in these cells.

DISCUSSION

The side effects reported to occur during cobalt therapy and the results obtained from animal experiments can be very easily explained on the basis of intoxication. It is very difficult to find any other general explanation for such phenomena as the disturbance of growth, development of goiter, claudication, and angina pectoris, and inhibition of tumor growth and vomiting observed in the therapy of human beings. The animal experiments give strong support to this point of view also.

Observations that the polycythemic effect of cobalt can be prevented by simultaneous administration of simple substances necessary to normal metabolism indicate that cobalt interferes with some basic metabolic systems in the organism.

The increased urinary excretion of coproporphyrin by the rat after cobalt treatment can be regarded as a toxic reaction, comparable to the porphyrinuria observed in other heavy metal poisoning.

Cobalt did not produce this reaction in doses of 0.25 mg. daily; a dose of 1 mg. was needed. Because the rats in both groups behaved quite similarly in every other respect, it can be assumed that other systems are involved which are more sensitive than porphyrin metabolism.

The reduction in weight of the cobalt-treated animals, adrenal hypertrophy, and reactive changes in the spleen are nothing but manifestations of stress, i.e., intoxication.

The present evidence we have of the toxic effects of cobalt put the value of cobalt as an antianemic factor under suspicion. It is very possible that cobalt accelerates erythropoiesis at the expense of tissue anoxia, and thus causes more damaging than beneficial effects. If cobalt is necessary in normal metabolism as a trace element, its amount must be minimal. In any case, some proof of the necessity of cobalt must therefore be demonstrated before its use in the treatment of anemias can be generally accepted.

SUMMARY

Cobalt, administered daily in a dose of 1 mg. subcutaneously, produces porphyrinuria in the rat. Increased amounts of coproporphyrin in urine were observed even after the first injection.

The toxicity of cobalt also manifests itself as a general stress reaction in the animals, as is indicated by the reduction in body weight, adrenal hypertrophy, and reactive changes in the spleen.

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ERYTHROCYTE ELECTROLYTES IN MUSCLE DISEASE

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MUSCULAR dystrophy is a heritable disease characterized by progressive weakness and wasting of muscle. Residual muscle from patients with muscular dystrophy characteristically shows a decreased level of intracellular potassium and increased intracellular sodium when compared with muscle from apparently healthy persons.[1, 2] Disturbed electrolyte concentrations have been found in circulating erythrocytes in a variety of disease states which reflect the abnormal intracellular sodium and potassium concentrations in muscle.[3, 4] Therefore, we have measured erythrocyte electrolytes in a series of patients with diseases of the muscle.

MATERIALS AND METHODS

Blood samples were obtained from 17 apparently healthy adults, 18 apparently healthy children, 30 patients with pseudohypertrophic muscular dystrophy, and 23 patients with other diseases of muscle. All had been fasting overnight. Ten λ of a 5 per cent solution of sodium-free ammonium heparin* per 10 ml. blood was used as anticoagulant.

Red cells were isolated for analysis by means of a new red cell separator designed after a suggestion of Dr. Gilbert Ling. A diagram of the separator is shown in Fig. 1. The stainless steel insert was pushed into a 20 ml. Lusteroid centrifuge tube. Four 0.075 by 0.050 inch grooves were machined into the barrier which separated the centrifuge tube into 2 compartments. Approximately 5 ml. heparinized blood was put into the upper compartment and the tube was centrifuged in a refrigerated centrifuge at 6,500 G for 30 minutes. The red cells moved through the grooves in the barrier and into the lower compartment; at the end of centrifugation the lower compartment was filled with erythrocytes. The serum and red cells remaining in the upper compartment were removed by suction and the Lusteroid tube was cut at the barrier with a penknife. The insert then was removed and the cells (approximately 1.25 Gm.) were left in the lower compartment at the end of the centrifuge tube.

Smears of the red cells showed them to be essentially free of leukocytes and platelets. The amount of trapped serum in the red cells was evaluated by means of radioiodinated human serum albumin. A known amount of I^{131}-albumin was added to 10 random blood samples. The greatest amount of trapped serum, computed from measurement of radioactivity in the separated red cells, was 1.39 per cent (volume per weight) with an average of 1.03 per cent. These figures may be higher than the actual trapped serum because some of the radioactivity of the separated erythrocytes represents albumin adsorbed on red cells.

An accurately weighed aliquot of red cells and a known amount of lithium chloride, which served as an internal standard, were diluted with water containing 0.01 per cent saponin. The sodium and potassium content was determined in an Advance Instrument flame photometer.

The electrolyte composition of erythrocytes has been shown to differ depending on the layer of packed red cells after centrifugation.[5] Although we have not attempted careful control of this variable, we did use the same quantity of blood (5 ml.) for each analysis.

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*Dr. James Merkel, Abbott Laboratories, North Chicago, Ill., provided us with a small quantity of sodium-free ammonium heparin.

TABLE I. ERYTHROCYTE SODIUM AND POTASSIUM IN NORMAL HUMANS AND PATIENTS WITH MUSCLE DISEASE

SUBJECTS	NUMBER OF CASES		ERYTHROCYTE ELECTROLYTES (mM/Kg.)	
	MEN	WOMEN	SODIUM	POTASSIUM
Normal men	17		13.61 ± 1.16	85.7 ± 3.0
Normal women		18	12.56 ± 1.29	88.6 ± 3.2
All normal individuals	17	18	13.09 ± 1.35	87.2 ± 3.4
Pseudohypertrophic muscular dystrophy patients	29	1	15.47 ± 2.18	90.9 ± 6.3
Fascio-scapulo-humeral dystrophy patients	2	3	13.05 ± 1.52	88.0 ± 4.2
Amyotonia congenita patients	4	6	14.01 ± 1.59	88.9 ± 5.0
Muscle atrophy, neurogenic patients	6	2	13.11 ± 1.10	91.8 ± 4.9

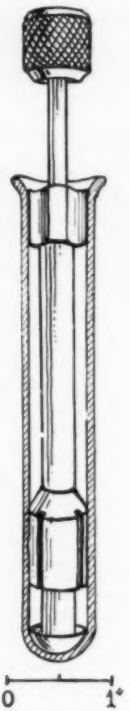

Fig. 1.—Red cell separator.

RESULTS AND DISCUSSION

The results are summarized in Table I. In apparently healthy control subjects the erythrocyte sodium content was 13.09 ± 1.35 millimoles per kilogram, and the potassium 87.2 ± 3.4 millimoles per kilogram. Using 1.099 for the density of human erythrocytes,[6] the average erythrocyte sodium content is 14.31 milliequivalents per liter, and the average potassium 95.6 milliequivalents

per liter. Nichols and Nichols[3] reported the average red cell sodium in normal subjects to be 16.3 mEq. per L., and the potassium 104.5 mEq. per L. Solomon[7] obtained 9.2 mEq. per L. and 94.1 mEq. per L., respectively, for the average red cell sodium and potassium.

Examination of red cells from 30 patients with pseudohypertrophic muscular dystrophy (29 men and 1 woman) disclosed a sodium content of 15.47 ± 2.18 mM per kilogram and a potassium content of 90.9 ± 6.3 mM per kilogram. The sodium content of cells from dystrophic subjects is significantly higher ($P < 0.01$) than that of red cells of all normal males or of prepubertal males. The potassium content of these red cells, while not significantly different from that of normal males ($0.2 < P < 0.3$), is higher than in normal males. In contrast dystrophic muscle has a lower potassium content than normal muscle. Unfortunately, red cell water was not measured, and we do not know whether the differences in electrolyte content represents, in part, concentration of cell solids. While the mean potassium content of the erythrocytes from dystrophic patients was greater than the mean of normal males, the potassium-sodium ratio was 5.8, less than the potassium-sodium ratio of 6.3 for normal males or 6.7 for all normal individuals. The changes in the erythrocyte electrolytes in dystrophy are not nearly so great as those found in muscle. While both muscle and red cells show abnormalities of electrolyte content in pseudohypertrophic muscular dystrophy, it cannot be concluded from this study that the mechanism of the abnormality in these two tissues is the same.

In fascio-scapulo-humeral dystrophy, amyotonia congenita, and neurogenic muscle atrophy, the erythrocyte sodium and potassium showed no significant difference from those of the normal controls.

SUMMARY

A new method of separating erythrocytes is described. The mean erythrocyte sodium was 13.09 ± 1.35 mM per kilogram and potassium 87.2 ± 3.4 mM per kilogram in apparently healthy subjects. The red cell sodium of patients with pseudohypertrophic muscular dystrophy (29 males, 1 female) averaged 15.47 mM per kilogram. This was a significantly higher figure than that for the normal men or for all the normal individuals studied. The average potassium level of red cells of dystrophic subjects was 90.9 mM per kilogram. This figure, while not significantly different from that for the normal individuals, was higher in contrast to the figure for muscle potassium which is lower in dystrophy.

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STUDIES ON THE NORMAL SERUM PANAGGLUTININ ACTIVE AGAINST TRYPSINATED HUMAN ERYTHROCYTES

V. The Nonidentity of the Agglutinin With Serum Trypsin Inhibitor and the Components of Complement

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ROSENTHAL and Schwartz[1] noted that trypsinated erythrocytes would agglutinate reversibly with serum from the same blood specimen. Agglutination was shown to be dependent upon a heat-labile, nondialyzable, presumably protein agglutinin; agglutination dispersal was associated with a heat-stable, dialyzable substance of uncertain nature. From subsequent studies, it was ascertained that this normal autoagglutinin (NAA) could be absorbed and eluted.[2] Although possessing some antibody-like properties, NAA was found to be concentrated in Cohn fractions I and III,[3] was unrelated to cold agglutinin,[3] was present in the serum of patients with agammaglobinulinemia,[4] and was unchanged in titer from infancy to old age.[5] Such properties indicate that normal autoagglutinin is not an antibody.[4]

Hoyt and Zwicker,[6] on the basis of indirect evidence, considered NAA and serum trypsin inhibitor to be identical. The work of Dacie,[11] demonstrating that nonlytic components of complement were adsorbed with incomplete cold antibody to the surface of the erythrocyte, raised the possibility that NAA might be one of these components acting in a reversible manner. Studies presented here do not support either of these hypotheses.

MATERIALS AND METHODS

Blood Collection.—Blood, obtained from members of the hospital staff and patients with nonhematologic disorders, was defibrinated with glass beads.

Trypsination of Erythrocytes.—Trypsination was performed as described by Payne, Spaet, and Aggeler.[7]

Titration of NAA.—For use in the titration of NAA, trypsinated cells were diluted to approximately 2 per cent in normal saline. One-tenth milliliter quantities of serially diluted serum were incubated with 0.1 ml. of the cell suspension, centrifuged slowly for approximately 1 minute, and examined immediately for agglutination.

Preparation of an Eluate of NAA.—An eluate of NAA was prepared by a modification of the method described by Spaet and Ostrum.[2] Twelve 1 ml. aliquots of serum were absorbed twice with 0.1 ml. portions of packed, trypsinated erythrocytes. The agglutinated cells were washed twice with saline, pooled, and washed once again with saline. The agglutinated cells were dispersed with 3 drops of a 0.1 per cent solution of sodium cyanide[2]; 6 ml. of normal

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saline were added, the cell suspension centrifuged, and the supernatant containing an eluate of NAA was removed and dialyzed for 4 hours against three 2,000 ml. changes of normal saline to remove the cyanide. The eluate was concentrated by overnight dialysis against 250 ml. of 10 per cent dextran and was then tested for normal autoagglutinin and serum trypsin inhibitor. The NAA titer of the concentrated eluate was 1:8, that of the original serum was 1:32.

Serum Trypsin Inhibitor.—The semiquantitative technique of Payne and associates[7] was employed. This method measured the ability of varying dilutions of serum to inhibit the tryptic digestion on the gelatin surface of a standard x-ray film.

Titration of Complement.—The methods of Kabat and Mayer[9] were used. The sheep cell concentration was standardized with a Coleman Junior spectrophotometer. The cells were sensitized with 4 units of hemolysin* and kept no longer than 12 hours.

Complement Components.—The individual components of complement were removed from separate aliquots of serum as described.[9] Serum was rendered free of C′1 and C′2 by heating to 56° C. for 30 minutes. C′3 was removed by zymosan treatment at 37° C. C′4 was removed by incubation with NH_4OH and subsequent neutralization with HCl.

Each reagent was tested for complement inactivation, and anticomplementary activity.[9] No attempt was made to titrate the components of complement; it was merely ascertained that the reagent tested was free only of the specific component inactivated, by demonstrating complement activity upon appropriate reagent combination.

RESULTS

The Relationship of NAA to Serum Trypsin Inhibitor.—Two milliliters of serum with an initial NAA titer of 1:16 was absorbed 6 times with 0.4 ml. aliquots of packed, trypsinated erythrocytes from the same blood specimen. As a control, 2 ml. of serum was similarly absorbed with washed but untreated erythrocytes. NAA was tested in both absorbed sera and found to be absent in the specimen absorbed with trypsinated erythrocytes, but present to a 1:2 dilution in the specimen absorbed with the untreated red cells.

Serum trypsin inhibitor was measured in the unabsorbed serum, and the 2 absorbed specimens. The results are shown in Fig. 1. As can be seen, absorption of NAA from serum does not remove the serum trypsin inhibitor as measured here. The change in titer from that of the original serum can be attributed to dilution. Conversely, the NAA eluate was devoid of antitryptic actvity.

It has been shown that if serum is shaken with chloroform 90 per cent of its antitryptic activity is destroyed.[8] Five milliliters of serum was mixed with 0.5 ml. of chloroform and shaken for 2 minutes. The chloroform was removed by centrifugation and the serum NAA titer and serum antitryptic activity measured. The NAA titer of the original serum was 1:32; that of the chloroform-treated serum was also 1:32. The serum antitryptic titer of the original serum was 1:16; that of the chloroform-treated serum was 1:1.

The Relationship of NAA to Serum Complement.—Aliquots of serum from which C′1 and C′2 had been removed by heat had the same NAA titer as that of the original serum. The same was true of specimens lacking C′3 activity. When the serum was rendered free of C′4 by NH_4OH treatment and subsequent neutralization with HCl, it was found that the NAA titer was consistently

*Obtained from the New York City Department of Health.

reduced from an original value of 1:32 to 1:8. However, treatment of an aliquot of serum with NH_4Cl also reduced the titer of NAA despite the fact that there was no change in complement titer. It was also observed that the autoagglutination of trypsinated cells with serum could be dispersed by dropwise addition of NH_4Cl. The effect of the NH_4^+ ion upon the autoagglutination of trypsinated erythrocytes was clearly unrelated to C'4 activity.

Two milliliters of serum were absorbed 6 times with 0.4 ml. of packed, trypsinated erythrocytes; a similar aliquot of serum was absorbed 6 times with packed, untreated cells. The NAA titer and the complement titer of both specimens were measured. Table I demonstates that removal of NAA from serum does not affect its complement titer. The slight drop seen can be attributed to dilution.

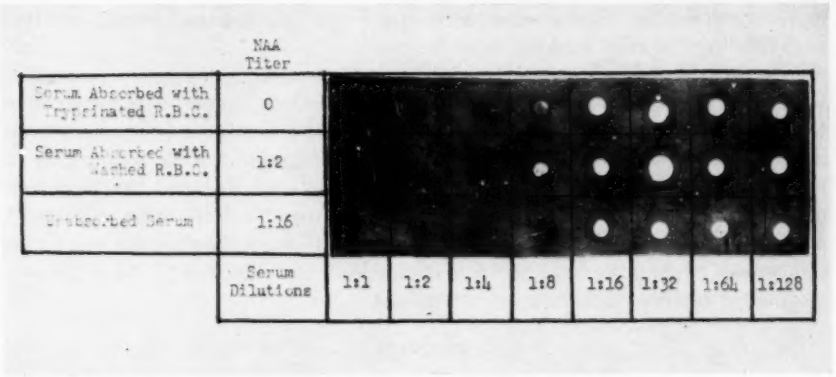

Fig. 1.—Inhibition of trypsin by serum absorbed with trypsinated erythrocytes.

TABLE I. NAA AND COMPLEMENT TITER OF SERUM ABSORBED WITH TRYPSINATED OR WASHED ERYTHROCYTES

	NAA TITER	COMPLEMENT TITER*
Unabsorbed serum	1:32	25
Serum absorbed with trypsinated erythrocytes	0	12.5
Serum absorbed with washed erythrocytes	1:4	12.5

*Expressed as units per milliliter.

DISCUSSION

The existence of erythrocyte panagglutinins in normal serum has been recognized for many years. The first of these to be described was the T-agglutinin which was shown to be active against all human erythrocytes that had been exposed to bacilli of the Corynebacterium or Vibrio genus.[12] The agglutinin is present in all sera save that of cord and neonatal blood. A panagglutinin active against the periodate-treated erythrocyte has been described and shown to be unrelated to T-agglutinin.[14] Available evidence indicates that NAA is an analogous panagglutinin active against the trypsinated human erythrocyte.

The physiologic role of the panagglutinins remains completely uncertain. In fact, it has been questioned whether the panagglutination reaction of these

serum constituents is at all related to their in vivo function. The suggestion[6] that the agglutination of trypsinated cells by NAA is merely a secondary property of normal serum trypsin inhibitor activity reacting with trypsin absorbed onto the erythrocyte would seem to be excluded by our data. Similarly, a reflection of complement activity may be ruled out. Serum properidin is inactivated at 56° C., and can also be excluded from consideration.[10]

If the panagglutinins do play a physiologic role, it might be expected that some deviation of function or concentration would be expected in the various disease states. To date, none has been detected. Two patients, with serum containing a high titer panagglutinin active only against trypsinated cells, have been described. In one[7] an acquired hemolytic anemia resulted; in the other[15] no corollary clinical state ensued. The in vitro properties of these 2 agglutinins differed from one another and from NAA, and each would seem to be a separate and distinct entity. Similar cases involving T-agglutination and activity against periodate-treated cells have not been described.

Neter[13] has raised the rather intriguing speculation that such panagglutinins may render erythrocytes damaged by infectious agents more susceptible to phagocytosis and subsequent destruction by the reticuloendothelial system. Such a mechanism would provide a plausible explanation for the diminution in red cell survival observed. In support of this, it may be noted that approximately 20 patients with polyagglutinable erythrocytes have been reported[16] principally in the course of infectious disease. If such should turn out to be the correct explanation, the panagglutinins will cease to occupy their present position of interesting laboratory curiosities.

SUMMARY

The normal serum panagglutinin active against trypsinated erythrocytes has been shown to be distinct from normal serum trypsin inhibitor and components of the complement complex. For the present, it must remain with the T-agglutinin and the agglutinin active against periodate-treated red cells, i.e., a panagglutinin present in normal serum which is active against an altered erythrocyte surface.

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THE EFFECT OF SYNTHETIC ANGIOTONIN UPON THE HEART OF THE INTACT DOG

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ANGIOTONIN (hypertensin) has been studied thoroughly in respect to the many facets of its action.[1] It is the product of the reaction of renin and hypertensinogen. This substance was recently synthesized,[2] and its identity with naturally occurring angiotonin established.[3] While it is known that the substance is a powerful vasoconstrictor, its action upon the heart has been studied only in the isolated heart preparation.[4]

As part of a study of the coronary hemodynamics and efficiency of the intact dog heart, the effects of angiotonin were examined, and are here presented.

MATERIALS AND METHODS

Ten dogs, apparently in good health, were used. They were not selected by breed, age, or sex, and weighed between 15 and 28 kilograms. The animals were premedicated with morphine sulfate, 3 mg. per kg., given subcutaneously. After 1 hour, Dial-urethane-Nembutal (50:50 mixture), was given intravenously in a dose of 0.25 ml. per kg.

By fluoroscopic control, cardiac catheters were then placed in the coronary sinus, pulmonary artery, and right atrium of the animal. A Cournand needle was placed percutaneously in the femoral artery.

The expired air was collected in a Tissot Spirometer by way of a cuffed endotracheal tube connected to a system of one-way flutter valves.

One hour following the intravenous anesthetic, the animal was in a steady state of anesthesia and had a sinus arrhythmia. At this time cardiac output by the Fick principle was measured, followed shortly by measurement of coronary blood flow by the nitrous oxide saturation method. The general methodology has already been reported from this laboratory.[5, 6] In this study the anesthetic was different. A previous control study using an infusion of saline was made, and the reproducibility of the method is shown in the appendix.

On completion of the control measurements, angiotonin (as the synthetic octapeptide*), was given in a dose of 0.3 mg. dissolved in 25 ml. of saline. This was administered by mechanical pump into the right atrium at a speed of 1 ml. per minute. Cardiac output was estimated after 5 minutes of infusion, and coronary blood flow as soon as practicable thereafter. The total infusion of angiotonin averaged 20 minutes, and the average dose given was 2.5 mg. On completion of the study, the dog was killed and the heart rapidly excised. Following injection of the left coronary arteries with indigo carmine, the stained area was excised and weighed wet. This value was used to calculate cardiac efficiency by the accepted method.[7]

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TABLE I. THE EFFECT OF ANGIOTONIN UPON GENERAL METABOLISM

FACTOR	CONTROL	EXPERIMENT
Volume of respiration (L./min.)	3.0 ± 0.7	3.6 ± 1.2
Respiratory rate per minute	13 ± 2	17 ± 1*
Oxygen consumption (ml./min.)	106 ± 22	110 ± 23
Carbon dioxide production (ml./min.)	94 ± 26	98 ± 27
Respiratory quotient	0.88 ± 0.08	0.88 ± 0.08
△ Arterial-mixed venous O_2 (Vol. per cent)	4.8 ± 1.0	5.4 ± 0.8
△ Mixed venous-arterial CO_2 (Vol. per cent)	4.1 ± 1.3	4.3 ± 0.9

Figures are means with S. D.
*Indicates "p" value 0.05 or less.

TABLE II. THE EFFECT OF ANGIOTONIN UPON SYSTEMIC AND PULMONARY HEMODYNAMICS

FACTOR	CONTROL	EXPERIMENTAL
Cardiac output (L./min.)	2.2 ± 0.6	2.0 ± 0.4
Heart rate per minute	77 ± 11	85 ± 20
Right atrial pressure (mean, mm. Hg.)	4.5 ± 1.8	3.9 ± 2.0
Femoral blood pressure (mean, mm. Hg)	113 ± 9	148 ± 10*
Pulmonary arterial blood pressure (mean, mm. Hg)	14 ± 1.8	14 ± 2.0
Left ventricular work (Kg. M./min.)	3.5 ± 0.8	4.1 ± 1.0*
Stroke left ventricular work (Gm./M./min.)	46 ± 12	54 ± 18
Right ventricular work (Kg. M./min.)	0.4 ± 0.1	0.4 ± 0.1
Calculated peripheral resistance (dynes sec. $cm.^{-5}$)	4,180 ± 1,018	6,045 ± 547*
Calculated pulmonary resistance (dyncs sec. $cm.^{-5}$).	488 ± 180	568 ± 275

Figures are means with S. D.
*Indicates "p" value 0.05 or less.

TABLE III. THE EFFECT OF ANGIOTONIN UPON CORONARY HEMODYNAMICS AND MYOCARDIAL METABOLISM OF O_2 AND CO_2

FACTOR	CONTROL	EXPERIMENTAL
Coronary blood flow (ml./100 Gm. myocard./ min.)	82 ± 19	86 ± 22
Coronary vascular resistance (units)	1.4 ± 0.28	1.8 ± 0.34*
Coronary flow/beat (ml.)	1.0 ± 0.15	1.1 ± 0.15
Coronary blood flow per left ventricular work (ratio)	24 ± 6	21 ± 5*
Coronary sinus O_2 content (vol. per cent)	4.8 ± 1.5	2.8 ± 0.9*
△ Arterial-coronary sinus O_2 (vol. per cent)	11.7 ± 1.8	14.5 ± 1.5*
Coronary sinus CO_2 content (vol. per cent)	58.3 ± 6.0	58.5 ± 6.0
△ Coronary sinus-arterial CO_2 (vol. per cent)	10.3 ± 1.6	12.2 ± 4.4*
Cardiac respiratory quotient	0.87 ± 0.07	0.84 ± 0.09
Cardiac metabolic rate-O_2 (ml./100 Gm. myocard./min.)	9.4 ± 1.8	12.4 ± 2.9*
Cardiac metabolic rate-CO_2 (ml./100 Gm. myocard./min.)	8.2 ± 1.4	10.6 ± 4.1
Index of efficiency (L. V. Work/$CMRO_2$)	0.38 ± 0.09	0.36 ± 0.03
Cardiac O_2 consumption† (ml./100 Gm./min.)	8.4 ± 1.8	11.1 ± 2.7*
"Mechanical" efficiency† (per cent)	20 ± 3.6	19 ± 4.5*

Figures are means with S. D.
*Indicates "p" value 0.05 or less.
†Derived from weighed left ventricle.

RESULTS

These are presented in the tables as group means with standard deviation. Statistical (not physiologic) significance was accepted at the 5 per cent level.

Within 1½ minutes of beginning the infusion, an increase in mean femoral pressure was observed, which was maintained throughout the experimental period.

TABLE IV. CONTROL GROUP OF TEN DOGS

FACTOR	CONTROL	AFTER SALINE	FACTOR	CONTROL	AFTER SALINE
Oxygen consumption (ml./min.)	108 ± 25	112 ± 20	Coronary sinus oxygen (vol. per cent)	4.1 ± 1.8	4.7 ± 1.3
Carbon dioxide production (ml./min.)	97 ± 24	103 ± 18	△ Arterial-coronary sinus O_2 (vol. per cent)	12.1 ± 0.7	12.9 ± 1.6
Respiratory quotient	0.89 ± 0.04	0.91 ± 0.07	Coronary sinus carbon dioxide (vol. per cent)	57.8 ± 5.8	56.5 ± 5.9*
Arterial oxygen (vol. per cent)	16.7 ± 1.6	17.5 ± 1.8	△ Coronary sinus-arterial CO_2	10.6 ± 0.6	11.3 ± 1.5
Mixed venous oxygen (vol. per cent)	11.9 ± 2.3	13.2 ± 2.1	Cardiac respiratory quotient	0.88 ± 0.04	0.87 ± 0.02
Mixed venous carbon dioxide (vol. per cent)	52.4 ± 6.0	49.8 ± 7.0*	Coronary flow (ml./100 Gm./min.)	90 ± 16	87 ± 17
Arterial carbon dioxide (vol. per cent)	48.2 ± 6.0	45.8 ± 7.0	Coronary vascular resistance (units)	1.3 ± 0.4	1.3 ± 0.3
Cardiac output (L./min.)	2.3 ± 0.8	2.7 ± 0.6	Cardiac metabolic rate—O_2 (ml./100 Gm./min.)	10.9 ± 1.9	11.1 ± 2.3
Heart rate per minute	87 ± 12	95 ± 14	Cardiac metabolic rate—CO_2 (ml./100 Gm./min.)	9.6 ± 1.6	9.7 ± 2.1
Femoral blood pressure (mean, mm. Hg)	116 ± 17	112 ± 16	Index of efficiency (L. V. work ÷ $CMRO_2$)	0.35 ± 0.14	0.38 ± 0.11
Pulmonary arterial blood pressure (mean, mm. Hg)	13 ± 3	14 ± 2	Hemoglobin (Gm. per cent)	13.8 ± 1.3	14.4 ± 1.5
Left ventricular work (Kg./M./min.)	3.7 ± 1.1	4.1 ± 1.2	Hematocrit (per cent)	43 ± 4	45 ± 4*
Right ventricular work (Kg./M./min.)	0.44 ± 0.20	0.50 ± 0.1			
Peripheral resistance (dynes/cm.$^{-5}$/sec.)	4,336 ± 1,380	3,488 ± 756			
Pulmonary resistance (dynes/cm.$^{-5}$/sec.)	477 ± 119	434 ± 115			

Figures are means with standard deviation.
*"p" value of change 0.05 or less.

As shown in Table I, few changes are recorded in the broad area of gaseous exchange and oxygen extraction; only the respiratory rate is significantly increased.

The findings in Table II suggest that the cardiac output remains at control levels, as does the heart rate. Left ventricular work and peripheral resistance (calculated) show a definite increase.

In Table III it is shown that the coronary blood flow is maintained at control value. The significant decrease in coronary sinus oxygen content is associated with an increase in cardiac oxygen extraction and metabolic rate for oxygen. Similar trends are found for carbon dioxide metabolism. Cardiac efficiency is well maintained.

DISCUSSION

In the dose used, angiotonin exerted an effect on the blood pressure throughout the experimental period. Continuous infusion was chosen in order to avoid the tachyphylaxis which may occur.[1] This facet of its action is well known. The identity of action of natural and synthetic angiotonin has already been established.[3]

In keeping with the findings of Middleton and Wiggers[8] no real change in cardiac output was found. In the present study, no change in right atrial mean pressure was found. This contrasts with results of the study already cited,[8] and may be related to the relatively smaller doses used.

Angiotonin has been said to raise pulmonary arterial pressure.[9, 10] This did not occur in the present study, for reasons not readily obvious; though the "open-chest" preparation of some previous studies may be a partial explanation for this discrepancy. The rise in peripheral resistance compares well with that observed in previous studies.[8]

The coronary blood flow values of the group are unchanged, although in the face of an increased perfusion pressure the calculated coronary vascular resistance must increase. This maintenance of flow is indicative that angiotonin does not constrict the coronary bed more than the peripheral vessels (cf. increase of resistance at 29 per cent and 44 per cent, respectively).

The decrease in coronary sinus oxygen content implies a higher extraction rate for oxygen, and thus, with the maintenance of coronary blood flow, results in an increased oxygen consumption by the heart.

An increased oxygen consumption by the heart is found in certain circumstances; as in induced tachycardia,[5] increased cardiac output,[11] and increased left ventricular work.[12] In the present study, the heart rate was maintained at control values as was the cardiac output, so that the increase in left ventricular work may be the strongest association. Correlation coefficients for rate, and cardiac output are not significant, but that for left ventricular work is significant ($R = 0.8$). These correlations are of course not necessarily causal, but under the circumstances are certainly suggestive of a causative relation. The maintenance of cardiac efficiency at control levels is the natural mathematical result of the increase in left ventricular work.

In general the coronary hemodynamic results are surprisingly comparable to those found in the isolated heart.[4]

SUMMARY

In the dosage used, and the circumstances of the study presented, synthetic angiotonin octapeptide increased systemic arterial pressure, peripheral resistance, and left ventricular work. Coronary blood flow was maintained with an increase in cardiac oxygen consumption.

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CRANBERRY JUICE AND THE ANTIBACTERIAL ACTION OF HIPPURIC ACID

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THE results of treatment of chronic infections of the urinary tract are often unsatisfactory. Various folk remedies are reputed to give symptomatic relief in these infections. The possibility was entertained that antibacterial agents might be present in such remedies, and thus account for the reputed benefit. Cranberry juice has been widely used in the Cape Cod area of Massachusetts in treatment of infections of the urinary tract without objective data concerning its usefulness.

Two lines of evidence suggested that an investigation of the therapeutic value of cranberries might be of interest. The first was the demonstration that the feeding of cranberries (the berry of *Vaccinium macrocarpon*), or of cranberry juice, increased the urinary excretion of hippuric acid. In 1923, Blatherwick and Long[1] fed 350 Gm. of cranberries to a human subject and reported that the urinary excretion of hippuric acid rose by 4.74 Gm. during the next 24 hours; the increased excretion of hippuric acid was accompanied by a decrease in urine pH from 6.3 to 5.3. Ten years later, Fellers and associates[2] confirmed the finding that the urinary content of hippuric acid increased after the feeding of cranberries although changes in urinary pH did not accompany the increased excretion of hippuric acid. The authors suggested that the chief precursor to hippuric acid is quinic acid (Fig. 1) since benzoic acid is present in cranberries in amounts too small to account for the large excretion of hippuric acid.

Although the conversion of quinic acid to benzoic or hippuric acid in man has been a matter of controversy for many years,[3, 4] several investigators have confirmed the original observation of Lautemann[5, 6, 7, 8] that feeding levoquinic acid to man results in increased excretion of hippuric acid in the urine during the succeeding 24 hours. Although certain bacteria have the ability to aromatize quinic acid and related compounds, mammalian tissues have not been shown to have this ability. Whether the aromatization of quinic acid in man is carried out by the bacterial flora of the intestine or by mammalian cells is a problem requiring further study.

A second line of evidence suggesting that substances from cranberries might have antibacterial activity was the structural similarity of hippuric acid to

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mandelic acid, which inhibits bacterial multiplication. The antibacterial action of several organic acids, such as beta-hydroxybutyric, mandelic, lactic, and acetic acids has been known for many years. Rosenheim initially suggested that mandelic acid would be a useful urinary antiseptic because in urine it might be possible to maintain the degree of acidity necessary for its bacteriostatic activity.[9] Hippuric acid was studied briefly by Rosenheim who found that a 1 per cent solution at pH 5.5 was necessary to kill suspensions of *Escherichia coli*. No comparisons with mandelic acid on the basis of molar effectiveness were made.

The present study was undertaken to investigate the antibacterial activity of hippuric acid in vitro, and to determine the effect of ingestion of cranberry juice on urinary acidity and hippuric acid excretion.

MATERIALS AND METHODS

The cranberry juice used in these studies was commercial cranberry juice "cocktail," consisting of one part juice from pressed cranberries and 2 parts water, with added sugar. One liter of juice can be pressed from 1,500 Gm. of cranberries. The pH of cranberry juice "cocktail" is 2.5 to 3.0. In certain studies undiluted sweetened cranberry juice, known in the industry as "Cran," was used. Hippuric acid was administered orally as the chemically pure compound in gelatin capsules or as an aqueous suspension.

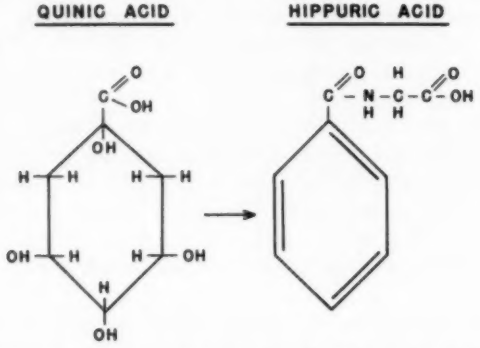

Fig. 1.—Structural formulas of quinic acid and hippuric acid. Quinic acid is found in cranberries, apples, pears, apricots, peaches, etc.

The subjects in these studies ate their normal diets. All urine specimens were refrigerated immediately after collection. The pH of the urine was determined with indicator paper that was accurate to 0.1 pH unit,* or with the Astrup pH meter. Venous blood was obtained under oil for determination of the pH and bicarbonate content by the Astrup method.[10] Titratable acidity of the urine was determined by titration of 10 ml. aliquots of undiluted urine to pH 7.4 with 0.3 N NaOH using the Astrup pH meter.

Urinary organic acids were measured by a modification of the method of Van Slyke and Palmer.[11] To a 50 ml. aliquot of urine was added 1 Gm. of calcium hydroxide. The mixture was agitated at intervals of 15 minutes, filtered, and 20 ml. of filtrate was titrated from pH 7.0 to pH 2.5 with 1 N HCl, using the Astrup pH meter. Urinary ammonia was measured by the microdiffusion method of Seligson and Seligson.[12] Hippuric acid in the urine was determined by ascending paper chromatography, according to the method of Gaffney[13] as modified by Armstrong.[14]

*Oxyphen paper (J. Einstein Company, Forest Hills 75, N. Y.).

Studies of bacteriostasis were conducted with solutions of hippuric acid in urine or in casein hydrolyzate broth (2 per cent casein hydrolyzate, 0.3 per cent yeast extract, and 0.5 per cent Na_2HPO_4). These solutions were adjusted to the desired pH with a few drops of concentrated HCl or NaOH, heated at 60° C. for 1 hour, and the pH again adjusted for the slight changes occurring after heating. Aliquots of 9.9 ml. were pipetted into sterile tubes and again heated at 60° C. for 1 hour. After the second heating, the pH was not more than 0.05 unit from the original value. Random samples of these solutions were tested for sterility; no growth was ever obtained.

The 9.9 ml. aliquots containing hippuric acid were inoculated with 0.1 ml. of appropriate dilutions, in nutrient broth, of overnight broth cultures of the desired microorganisms. The inoculum contained 10^3 to 10^5 bacteria. The inoculated tubes were incubated for 24 to 48 hours at 37° C. and aliquots were removed at intervals for colony counts, which were performed in the usual manner.

RESULTS

Excretion of Hippuric Acid After Ingestion of Cranberry Juice.—When cranberry juice was consumed in large amounts by normal subjects, there were slight or insignificant changes in urinary pH and small increases in the titratable acidity of the urine (Table I). The organic acid excretion increased significantly in all cases. Assuming all of the increased excretion of organic acid to be hippuric acid, the concentration of hippuric acid was only occasionally increased by as much as 0.02 M. Since 3 to 4 Gm. of hippuric acid was usually excreted after the juice had been ingested, the failure to achieve higher concentrations in the urine was due to the increased volume of urine. As will be demonstrated later, 0.02 M is the minimal concentration of hippuric acid that is bacteriostatic for *E. coli* at pH 5.0.

The blood pH of one subject (No. 3) was studied during the time that he was receiving 1,500 ml. of cranberry juice cocktail on each of 3 successive days. The blood pH was not significantly altered by this experience.

TABLE I. EFFECT OF INGESTION OF CRANBERRY JUICE ON TITRATABLE ACIDITY AND ORGANIC ACID CONTENT OF URINE

SUBJECT	AGE	DAY	CRANBERRY JUICE INGESTED (ML.)	URINE ANALYSES: pH URINE	URINE ANALYSES: TITRATABLE ACIDITY (mEq./24 HR.)	URINE ANALYSES: ORGANIC ACIDS (mEq./24 HR.)	Δ ORGANIC ACID* PER 24 HOURS (GM.)	Δ ORGANIC ACID* PER 24 HOURS (M)
1.	24	1	0	6.4	16.9	49.9	—	—
		2	1,200	6.2	21.5	68.1	3.3	0.014
2.	24	1	0	6.5	14.7	72.6	—	—
		2	1,200	6.4	19.1	78.4	1.0	0.004
3.	24	1	0	5.9	32.9	66.8	—	—
		2	1,500	6.0	34.8	75.0	1.5	0.008
		3	1,500	5.6	35.3	92.6	4.6	0.021
		4	1,500	5.6	37.3	90.2	4.2	0.020
4.	28	1	0	6.2	15.8	74.6	—	—
		2	4,000	6.3	28.0	94.1	3.5	0.006
5.	25	1	0	6.2	—	—	—	—
		2	600†	5.7	—	—	3.1	0.016

*Calculated on the assumption that all of the increased excretion of organic acid is due to hippuric acid.

†Undiluted cranberry juice ("Cran"), equivalent to 1,800 ml. of the cranberry juice "cocktail" that was consumed by the other subjects.

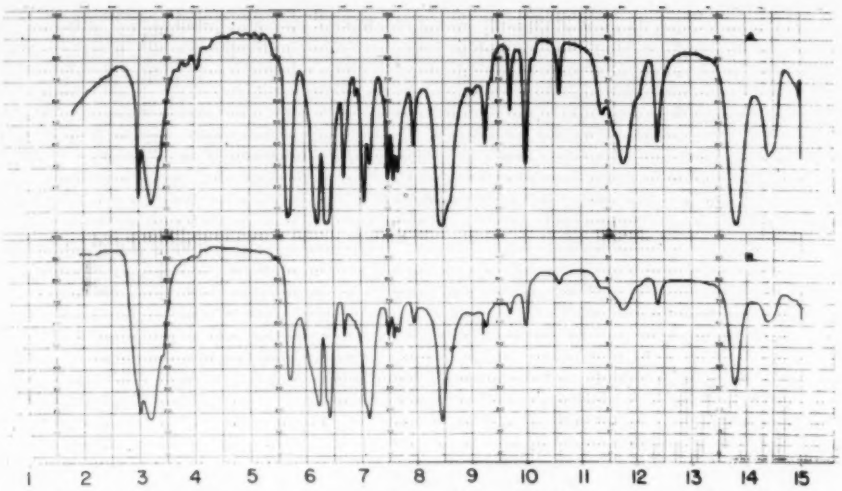

Fig. 2.—Infrared absorption spectra of pure hippuric acid (upper curve, *A*) and eluate of paper chromatographic zone of urine corresponding to hippuric acid (lower curve, *B*). Urine obtained after ingestion of cranberry juice (see text). Solvent: methanol. Eluate extracted once with petroleum ether before infrared absorption spectrum was obtained.

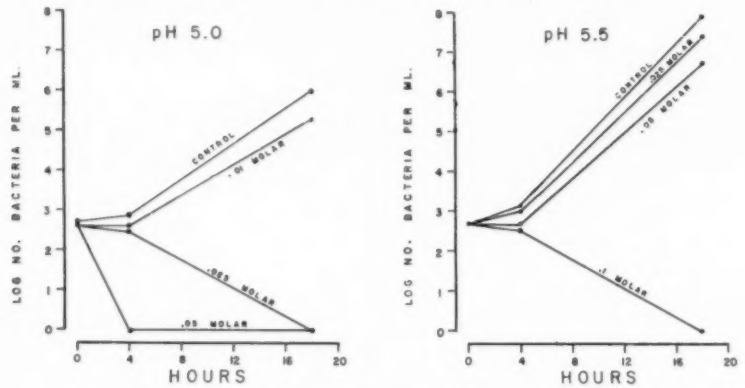

Fig. 3.—Effect of added hippuric acid on growth of *E. coli* in urine.

The hippuric acid content of several urines was determined chromatographically. In all instances, the increases in hippuric acid corresponded closely with the increases in organic acid as determined by titration. Definitive identification of the increased acid as hippuric acid was made by detailed studies of urine from Subject No. 5. The zones corresponding to hippuric acid were obtained from his urine by paper chromatography. These zones were cut out, eluted in methanol, and compared by infrared and ultraviolet spectrophotometry with samples of pure hippuric acid. The curves obtained were essentially identical (Fig. 2).*

*We are indebted to Dr. S. Kushner of the Lederle Laboratories Division of American Cyanamid Company, for these analyses.

The urines obtained before and after the ingestion of cranberry juice were tested for bacteriostatic action against a freshly isolated strain of *E. coli*. None of the urine samples possessed antibacterial activity at the pH at which they were voided. When the pH was adjusted to 5.0, only those samples containing at least 0.02 M hippuric acid were bacteriostatic.

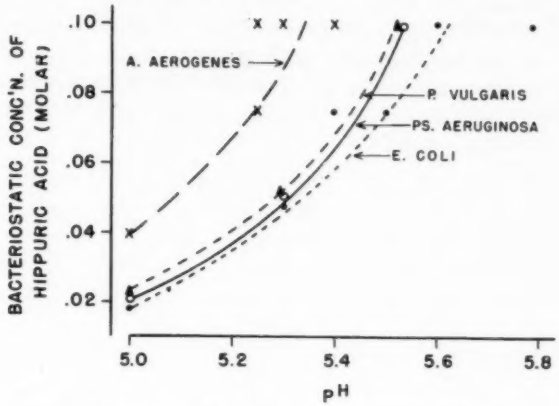

Fig. 4.—Bacteriostatic action of hippuric acid on common urinary tract pathogens.

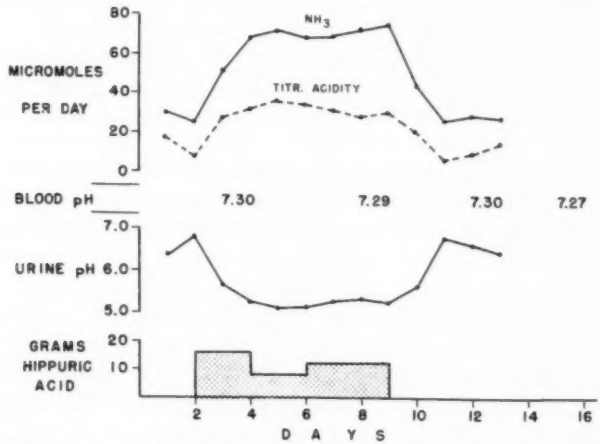

Fig. 5.—Effect of oral hippuric acid on urine pH, titratable acidity, and NH_3 excretion in a normal subject.

These studies show that cranberry juice is not an effective acidifier of the urine in normal subjects, but that it does increase the hippuric acid content of the urine. However, the increased content of hippuric acid is rarely sufficient for bacteriostasis at pH 5.0, even after large amounts have been ingested.

Antibacterial Action of Hippuric Acid in Vitro.—Solutions of hippuric acid at varying molar concentrations were prepared as previously described,

inoculated with 10^3 to 10^5 bacteria, and incubated. Aliquots were withdrawn at intervals for colony counts, and the results are shown in Fig. 3.

At pH 5.0, the minimal inhibiting concentration for *E. coli* was 0.025 M hippuric acid in this experiment. At pH 5.5, 0.1 M hippuric acid was required. More detailed studies showed that the minimal bacteriostatic concentration for two strains of *E. coli*, one strain of *Proteus vulgaris* and one of *Pseudomonas aeruginosa* was 0.02 M hippuric acid at pH 5.0. For two strains of *Staphylococcus aureus* the minimal bacteriostatic concentration was 0.03 M and for 10 strains of *Aerobacter aerogenes* it was 0.04 M at pH 5.0 (Fig. 4). This figure also shows that the concentration of hippuric acid required for bacteriostasis increases with increasing pH.

Since the pKa (the pH at which the number of ionized and unionized molecules in solution is equal) for hippuric acid is 3.8, the number of unionized molecules decreases as the pH increases above 3.8. The concentration of undissociated hippuric acid molecules at the minimal bacteriostatic concentrations of total hippuric acid was therefore calculated (Table II). Despite the five-

TABLE II. EFFECT OF pH ON BACTERIOSTATIC CONCENTRATION OF HIPPURIC ACID FOR E. COLI

	MINIMAL BACTERIOSTATIC CONCENTRATION	
pH	TOTAL HIPPURIC ACID mM.	UNDISSOCIATED HIPPURIC ACID mM.
5.0	20	0.76
5.3	50	0.98
5.4	75	1.17
5.5	75	0.94
5.6	100	0.91
5.7	100	0.70

fold increase in total acid necessary for bacteriostasis as the pH increased from 5.0 to 5.7, the minimal bacteriostatic concentration of undissociated hippuric acid was found to be approximately the same at the different pH values. This suggests that the effective molecule for bacteriostasis is the unionized one.

Effect of Ingestion of Hippuric Acid.—Hippuric acid in doses of 8 to 16 Gm. daily was given to 2 normal subjects in divided doses. The data obtained in the study of one subject are presented in Fig. 5 and are similar to the results obtained from the study of the other subject. The urine pH decreased to 5.0 to 5.4, and the titratable acidity and ammonia excretion more than doubled. These values were maintained during the 7 days that hippuric acid was given. Organic acid excretion increased, corresponding to excretion of about 80 per cent of the ingested acid. In one subject, the venous blood pH did not change significantly. In the other it fell from 7.35 to 7.25 after 4 days of ingestion of 16 Gm. of hippuric acid daily. Toxic effects were otherwise not observed. During the time of ingestion of hippuric acid, the concentration in the urine, calculated from the organic acid excretion, was 0.026 to 0.057 M, and all of the urines were bacteriostatic to *E. coli* at pH 5.0.

DISCUSSION

The observation that hippuric acid is bacteriostatic may explain why cranberries have been said to give relief in infections of the urinary tract. However, the data show that cranberry juice is an unreliable means for acidifying the urine, despite its own high acidity. Similarly, cranberry juice alone rarely increases the hippuric acid concentration in urine to the minimum (0.02-0.04 M) necessary for bacteriostasis of common urinary tract pathogens at pH 5.0.

Nevertheless, the demonstration of the antibacterial action of hippuric acid suggests further possibilities. Many foods other than cranberries contain precursors to hippuric acid, and excretion of hippuric acid in amounts as great as 4 Gm. per day has been reported after the ingestion of "ordinary" diets.[14] Such a concentration in normal urine volumes would be bacteriostatic at pH 5.0. This may explain the observation that pools of urine obtained from normal individuals are occasionally bacteriostatic at pH 5.0.[15, 16] The benefit sometimes claimed from the empirical use of various herbs, foods, and plant extracts in infections of the urinary tract may perhaps also be ascribed to the increased excretion of hippuric acid. However, the limiting effect of the pH of the urine might be expected to make such folk remedies inconstant in their effectiveness. For the same reason, it does not follow, as might be suggested, that herbivores should be free of infections of the urinary tract because of the high content of hippuric acid in their urines; the urines of herbivores are generally alkaline.

The similarity of the effect of hippuric acid to that of mandelic acid is apparent from the data presented. It is not yet known whether hippuric acid will be of therapeutic value in the management of patients with chronic urinary tract infections. Preliminary studies, however, suggest that the continued administration of hippuric acid may lead to suppression of bacteriuria if the pH of the urine can be maintained at sufficiently low values. The potential importance of control of urinary pH is demonstrated in the data that show that the concentration of hippuric acid necessary for bacteriostasis must be increased fivefold if the pH is allowed to increase from 5.0 to 5.6.

SUMMARY

Cranberry juice, given in maximal tolerable amounts to normal subjects, increases the acidity of the urine slightly and increases the hippuric acid content of the urine by several grams a day. Hippuric acid, in concentrations of 0.02-0.04 M, is bacteriostatic at pH 5.0 for the common pathogens of the urinary tract, and its activity decreases about fivefold as the pH is raised to 5.6. Only occasionally does cranberry juice contribute enough hippuric acid to the urine to achieve concentrations which are bacteriostatic at pH 5.0.

Hippuric acid, in doses of 12 to 14 Gm. per day, usually lowers the pH of the urine to 5.2 or less, and is excreted in sufficient concentration to induce inhibition of bacterial growth in urine at this pH.

The data suggest that the use of hippuric acid as a urinary antiseptic deserves further study.

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AIRWAY RESISTANCE STUDIES IN BRONCHIAL ASTHMA

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AN IMPORTANT ventilatory defect in bronchial asthma is a functional decrease in the caliber of bronchiolar lumen with a subsequent increase in resistance to airflow. Methods for studying this defect used by other investigators have been the timed vital capacity and mid-expiratory flow rate.[1-5] While these methods offer data which are useful in the clinical evaluation of patients, they do not specifically estimate the degree of airway narrowing; they are dependent upon patient cooperation; they are less sensitive than methods to be discussed.

The esophageal balloon technique for compliance studies has been used to measure the nonelastic or mechanical resistive forces during breathing in asthmatics.[6-8] This method, however, does not differentiate between airway resistance and tissue viscance factors, each of which may be abnormal in bronchial asthma.

DuBois, Botelho, and Comroe[9] have described a technique for measurement of airway resistance under the dynamic conditions of airflow without interference from the pressures necessary to overcome elastic and tissue viscance forces of the lung. The present study was undertaken to measure airway resistance in both symptomatic and asymptomatic patients with bronchial asthma. The effect of a nebulized bronchodilator, isoproterenol, upon airway resistance in these subjects was determined.

METHODS

Airway resistance was measured by means of a body plethysmograph, utilizing a technique similar to that described by DuBois, Botelho, and Comroe. The plethysmograph tank was made from a round, steel septic tank with curved bottom and top. An opening was cut into one side and a hinged, gasket-sealed, steel door assembly was attached. A Plexiglas insert in the door allowed close observation of the patient during the test. In final form the tank volume was approximately 700 liters. A sound-powered telephone allowed ready communication with the patient when the door was closed. A solenoid vent valve,† activated by a foot switch, allowed rapid venting of the tank to ambient pressures by the operator. Pressure changes within the tank were measured by means of a differential pressure transducer with a range of 0.01 pound square inch differential (p.s.i.d.).‡ One vent of the gauge was gasket-mounted into a hole in the side of the tank, the other vented into a ten gallon bottle which was open to ambient pressures. The latter arrangement minimized interference from extraneous convection currents in the room.

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†Asco Model 80306, Automatic Switch Company, Orange, N. J.

‡Statham Model PM 197 .01-350, Statham Laboratories, Inc., Los Angeles, Calif.

A heated, Lilly-type flowmeter with 400-mesh screen* was mounted inside the tank. Pressure drop across the screen was detected by a differential pressure transducer with a range of 0.05 p.s.i.d.† The flowmeter was mounted in series with a solenoid valve which allowed rapid electrical occlusion of the airway during testing. A third pressure transducer with a range of 0-5 mm. Hg‡ was mounted by means of a T tube to the proximal pressure vent on the flowmeter. This gauge was used to record mouth pressures when the airway was occluded. Outputs from the pressure transducers were fed into 3 strain gauge amplifier channels of a Sanborn Model 67 Polyviso. The paper record allowed continuous monitoring of all test procedures to insure that measurements were made within appropriate sensitivity ranges of all instruments. Each of the strain gauge outputs was fed into an oscilloscope§ fitted with a camera adapted for Polaroid photographic recording.‖ Output from the tank pressure transducer was fed onto the x-axis of the oscilloscope. An electrical relay was designed to switch y-axis information as follows: (1) when the flowmeter solenoid occluded the patient's airway, the mouth pressure gauge readings appeared on the y-axis and (2) when the flowmeter valve was open, the P-97 transducer with flow information was fed onto the y-axis.

Each pressure transducer was calibrated before each trial within the following scales: (1) Tank pressure change effected by 25 ml. air introduced into tank produced 1 inch deflection on the horizontal axis of the scope. (2) Mouth pressure gauge caused 1 inch deflection on the y-axis of scope when acted upon by a 7 cm. column of water from a water manometer. (3) Flow pressure gauge produced a 1 inch deflection on the y-axis of the scope with flow rate of 1.17 liters per second calibrated against a Fisher-Porter rotameter calibrated from 10 to 75 L. per minute flow.

The actual test procedure was as follows: The subject was seated inside the tank and the door sealed. A two to three minute wait was necessary to allow temperature and water vapor saturation within the tank to stabilize sufficiently so that pressure changes from these factors were minimized. The subject was instructed to pant shallowly at approximately 120 breaths per minute through the mouthpiece apparatus. The tank vent was closed and the airflow-plethysmograph pressure slope ($\dot{V}/P_p$) was photographed from the oscilloscope screen. While the patient continued the panting procedure, the flowmeter solenoid was suddenly closed and the mouth pressure-plethysmograph pressure slope (P_A/P_p) appeared on the scope. This slope was photographed as a double exposure on the Polaroid film. The entire procedure required less than 5 minutes for 3 or 4 complete records.

Airway resistance was then calculated by means of the formula $R = P_A/\dot{V}$. The following mathematical expression demonstrates how the 2 slopes may be divided to provide the necessary information to calculate resistance under the dynamic conditions of airflow:

$$R = \frac{P_A/P_p}{\dot{V}/P_p} = \frac{P_A}{P_p} \times \frac{P_p}{\dot{V}} = \frac{P_A}{\dot{V}}$$

R = resistance.
P_A = Δ alveolar pressure when airway is occluded.
P_p = Δ plethysmograph pressure.
$\dot{V}$ = Δ velocity of airflow.

From the calculated resistance a "mouthpiece factor" was subtracted to allow for resistance across the flowmeter-solenoid assembly, which is also measured by the test procedure. This factor was determined by calculating the mean air flow during the test from the Polyviso tracing and reading the correction factor from a chart graph previously constructed from a series of pressure drop measurements across the mouthpiece assembly.

*Technitrol Model 115-F-R, Technitrol Engineering Company, Philadelphia, Pa.
†Statham Model PM 97 .05-350, Statham Laboratories, Inc., Los Angeles, Calif.
‡Statham Model P 23 B, Statham Laboratories, Inc., Los Angeles, Calif.
§Dumont Model 401, Allen B. DuMont Laboratories, Clifton N. J.
‖Dumont Model 302 Camera, Allen B. DuMont Laboratories, Clifton, N. J.

On each patient at least 3 measurements were made which were required to check within 0.2 cm. H_2O per liter per second. An attempt was made to have the patient perform the breathing at functional residual capacity level. It was required that the slope P_A/P_p, which is directly related to lung volume, not change from test to test in any one individual. It has been shown in our laboratory and by others[10] that airway resistance is lowered by an increase in the degree of lung inflation; therefore, P_A/P_p must remain unchanged if results are to be comparable in the individual patient.

Pulmonary function in each patient was further evaluated by the following tests: (1) Maximum breathing capacity was measured by means of a Hans Rudolph high velocity valve, Douglas bag, and wet gas meter. (2) Lung volumes were determined by spirogram and a modification of the helium dilution functional residual capacity method of Meneely and Kaltreider.[11] Results were expressed as the ratio of residual volume to total lung capacity, RV/TLC. (3) Vital capacity and timed vital capacity were measured from spirographic tracings obtained on a Benedict-Roth 9-liter spirometer. (4) Arterial O_2 and CO_2 content were measured by the method of Van Slyke and Neill.[12] A modification of the Sendroy technique[13] for O_2 capacity was utilized. (5) Arterial pH was measured at 37° C. on a Beckman Model G pH meter with constant temperature type 290-31 blood electrode. (6) The Singer and Hastings nomogram[14] was used to calculate arterial PCO_2.

Normal subjects were selected laboratory personnel without history or findings of pulmonary disease. The patients studied in this group had a diagnosis of bronchial asthma based on history, physical examination, chest x-ray, and pulmonary function studies. All have been observed by us at some time during an acute attack of asthma. Except for W. H., W. N., C. H., P. S., and R. G., all have required hospitalization for management. Five patients, C. W., V. M., L. Mc., S. A., and E. R., had acute symptoms of asthma at the time of the study. No patient had received a bronchodilator drug or other medication for 6 hours prior to study. Timed vital capacities were measured immediately before initial airway resistance measurements. Later each subject was given nebulized isoproterenol. Timed vital capacity and airway resistance measurements were then repeated within 15 minutes.

RESULTS

The results of measurement of airway resistance in normal subjects are tabulated in Table I. Mean value for airway resistance was 1.25 cm. H_2O per

TABLE I. AIRWAY RESISTANCE IN NORMAL LABORATORY PERSONNEL

SUBJECT	AGE (YEARS)	A.R. CM. H_2O PER L. PER SEC.
1. R. C.	29	1.1
2. D. C.	31	1.5
3. L. H.	38	1.2
4. J. M.	24	1.3
5. C. A.	33	1.6
6. F. B.	34	1.0
7. R. V.	33	1.2
8. W. R.	33	1.1
9. C. L.	39	1.0
10. W. S.	30	1.5
Mean		1.25
Standard deviation		0.22

liter per second with a standard deviation of 0.22. Range of normal values was from 1.0 to 1.6 cm. H_2O per L. per sec. Table II lists clinical data and pulmonary function studies on patients with asthma. All subjects, regardless of symptomatology, had airway resistance values above the normal mean value plus 2 standard deviations.

TABLE II. TABULATION OF PULMONARY FUNCTION TESTS

PATIENT	AGE (YEARS)	TIME SINCE ATTACK	AIRWAY RESISTANCE CM. H_2O PER L. PER SEC.		VITAL CAPACITY PER CENT NORMAL	FIRST-SECOND VITAL CAPACITY		RESIDUAL VOLUME TOTAL LUNG CAPACITY	MBC		ARTERIAL O_2 PER CENT SATURATION	ARTERIAL PCO_2
			INITIAL	ISOPROTERENOL		INITIAL	ISOPROTERENOL		L.	PER CENT PRED.		
Symptomatic												
C. W.	21		4.3	4.3	78	56	56	37	63	48	91	30
V. M.	33		4.8	1.4	65	27	70	28			85	44
L. Mc.	31		9.6	3.8	42	52	51	47*	35	28	98	38
S. A.	48		6.5	4.2	75	39	52	33	80	65	95	37
E. R.	29		6.3	3.4	54	49	51	58*	38	30	91	39
Mean			6.3	3.4		45	56					
Asymptomatic												
B. L.	31	4 days	3.7	2.6	91	56	60	24			95	38
L. Mc.	31	4 days	5.3	3.6	67	67	70					
S. A.	48	7 days	3.6	3.5	78	66	63	33	80	65	95	37
A. J. S.	43	8 days	5.5	1.7	117	66	66	26	68	49	95	39
W. H.	24	1 yr.	2.3	2.1	73	49	49	20	76	53		
W. N.	32	1 yr.	2.1	1.3	121	74	77	20	118	85		
C. H.	25	14 days	2.0	1.7	83	53	70	33	65	50		
P. S.	29	60 days	2.4	1.9	95	65	67					
R. G.	24	30 days	5.1	1.5	77	84	81	28	93	70	95	36
Mean			3.6	2.2		64	68					

*Patient symptomatic when FRC level determined.

Airway resistance was reduced in most subjects of both the symptomatic and asymptomatic groups following nebulized isoproterenol 1:200. In only 6 instances was it reduced to the range of normal. Fig. 1 demonstrates these values and indicates graphically the change observed in each patient. Mean resistance for both groups before isoproterenol was 4.5 cm. H_2O per L. per sec. Following use of the bronchodilator, mean resistance fell to 2.6 cm. H_2O per L. per sec. The reduction is highly significant ($p < .01$).[15] Other tests of ventilatory function were often abnormal in both groups of asthmatics before

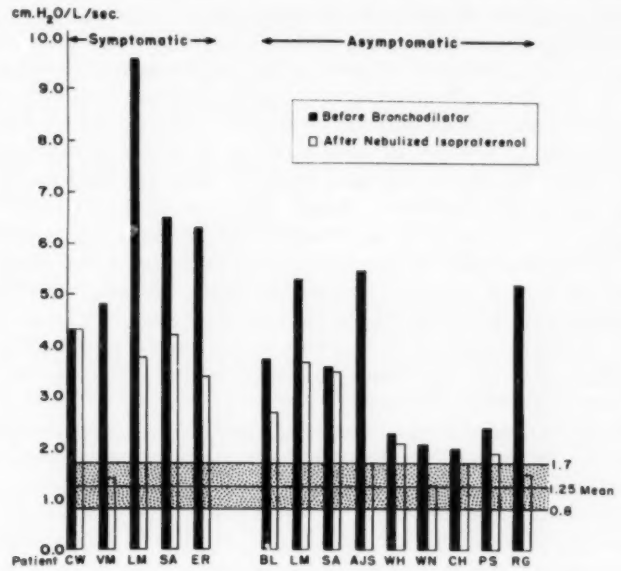

Fig. 1.—Airway resistance in patients with bronchial asthma.

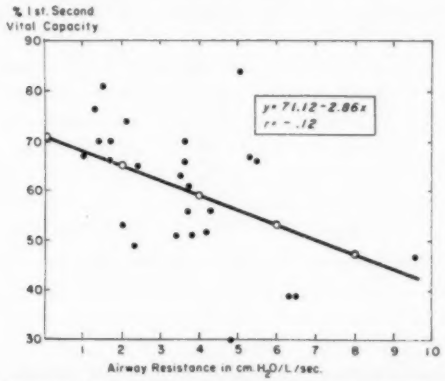

Fig. 2.—Relationship between first-second vital capacity and airway resistance in patients with bronchial asthma.

and after use of isoproterenol. Fig. 2 demonstrates the relationship between first-second vital capacity and airway resistance measurements. There was poor correlation between values of first-second vital capacity and airway resistance for each individual tested ($r = -0.12$).

DISCUSSION

Our data show that patients with bronchial asthma have increased airway resistance. Of particular interest is the finding that airway resistance is in the abnormal range whether patients are symptomatic or symptom free. Resistance values in this study are of similar magnitude to those reported by Marshall and DuBois[16] in their plethysmographic studies. Their patients were studied between attacks, but no statement is made of time relationship to acute symptoms.

McIlroy and Marshall report studies using dynamic compliance measurements to estimate the nonelastic resistive forces. They report a mean value of 14.4 cm. H_2O per L. per sec. in their series of 9 asthmatic patients.[8] Segal, utilizing a similar technique, obtained values of 12.68 cm. H_2O per L. per sec. These latter values are significantly higher than the values of airway resistance alone measured by DuBois or by us. Part of this difference can be attributed to resistance forces necessary to overcome increased tissue viscance. On the other hand, previously reported values for tissue viscance measurements in asthmatics have been less than 2.2 cm. H_2O per L. per sec.[16] These facts would imply that resistance has been overvalued previously or that the rate of breathing utilized in the plethysmographic technique results in underestimation of the airway resistance values.

In clinical use, nebulized isoproterenol has been an effective therapeutic agent in bronchial asthma. Other studies have shown that it changes ventilation, as manifested by improvement in maximum breathing capacities, and first-second vital capacity measurements.[1-4] Our studies show that at least part of the improvement in ventilation is specifically due to decrease in airway resistance, although resistance is seldom reduced to normal levels.

Previous studies have shown that pulmonary function is frequently abnormal in asymptomatic asthmatics.[17-19] Our data confirm this finding and show that at least part of the defect in function is due to increased airway resistance. There is overlap between resistance values associated with symptoms and those found in the asymptomatic patient. The correlation between airway resistance and first-second vital capacity, which previously has been used as an index of airflow resistance, is poor. Perhaps this is due to the fact that plethysmographic measurements as used in this study give values which represent a composite of inspiratory and expiratory resistances.

The actual long-term effects of increased airway resistance upon pulmonary structure are unknown. If continuous elevation of airway resistance hastens the progress of degenerative pulmonary disease, treatment of the asthmatic patient should be a continuous and vigorous attempt at maintenance of airway resistance as near to the normal range as possible. Only with the persistent administration of medication can resistance values approach normal range. Further long-term studies are necessary to elucidate the influence of airway resistance upon degenerative pulmonary disease.

SUMMARY

1. Airway resistance has been measured by means of a body plethysmograph and in normal subjects found to be 1.25 ± .22 cm. H_2O per L. per sec.

2. Airway resistance in patients with bronchial asthma ranged between 2.0 and 9.6 cm. H_2O per L. per sec.

3. Resistance values above the normal range were found in patients with bronchial asthma who had neither signs nor symptoms of their disease at the time tested.

4. Airway resistance in asymptomatic patients ranged between 2.0 and 5.5 cm. H_2O per L. per sec. Resistance in symptomatic patients ranged between 4.3 and 9.6 cm. H_2O per L. per sec.

5. Nebulized isoproterenol produced a significant reduction of airway resistance in both groups.

6. Poor correlation was found between airway resistance and first-second vital capacity.

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LABORATORY METHODS

A HYDRAULIC PRESSURE GENERATOR FOR TESTING THE DYNAMIC CHARACTERISTICS OF BLOOD PRESSURE MANOMETERS

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THE requirements for an ideal manometer system are that it have amplitude linearity, uniform frequency response, and constant time delay throughout the range of frequencies contained in pressure waves. Since the frequency content of blood pressure waves is not known accurately at the present time, it is desirable that a blood pressure manometer have the widest possible range of uniform response. The purpose of this paper is to describe a hydraulic pressure generator for testing the performance of blood pressure manometers when used either with needles or with catheters.

The requirements for the pressure generator are that it produce sinusoidal pressure waves which have a peak-to-peak amplitude of 50 mm. Hg and which are continuously adjustable in frequency from 0 to 1,000 cycles per second. The frequency settings must be accurate and stable. The pressure amplitude must be as constant as possible over the complete range. A monitoring manometer must be used to determine continuously the pressure actually being produced in the generator at all times. An additional practical requirement is that the entire apparatus be constructed from readily available and inexpensive materials in order that it may be made in any reasonably well-equipped machine shop.

METHODS AND MATERIALS

The requirements of relatively large pressure capability and accurate frequency setting suggest the use of an electromagnetically actuated piston or diaphragm driven by an electronic amplifier which is excited by a standard laboratory oscillator. The piston leakage problem and the requirement for very small fluid displacements suggest the diaphragm as the better choice. For optimum simplicity, ruggedness, and pressure capability, we have used the attractive force which a solenoid exerts on a diaphragm of magnetic material.

As shown in Fig. 1, the magnetic circuit consists of a field coil wound around a solid magnetic core which is set in a cup of magnetic material. A diaphragm also made of magnetic material covers the top of the cup, leaving a small air gap between the center of the diaphragm and the core of the coil. If we assume that the reluctance of the magnetic circuit is constant, the magnetic attractive force tending to pull the diaphragm down is proportional to the square of the current in the coil. However, if a large steady component of current and a small alternating component of current flow in the coil, the

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a. c. pull becomes approximately proportional to the a. c. component of current. The desirability of this arrangement is evident when it is realized that the coil may be connected directly in the plate circuit of a pentode or tetrode, thereby providing frequency response down to zero frequency. A pentode or tetrode is required because the a. c. plate current must be independent of frequency. The zero signal d. c. plate current of the pentode provides the necessary large steady component of current, and the small alternating component of plate current is provided by supplying the pentode grid with a sinusoidal voltage of suitable amplitude. The performance is similar to that of a magnetic headphone, with the permanent magnet of the headphone being replaced by the magnetization produced by the d. c. plate current of the tube.

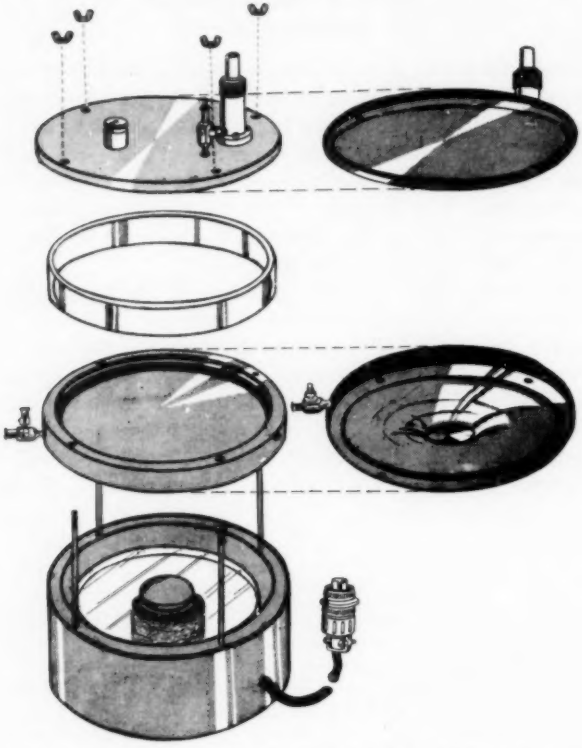


Fig. 1.—Exploded view of the hydraulic pressure generator showing mechanical details.

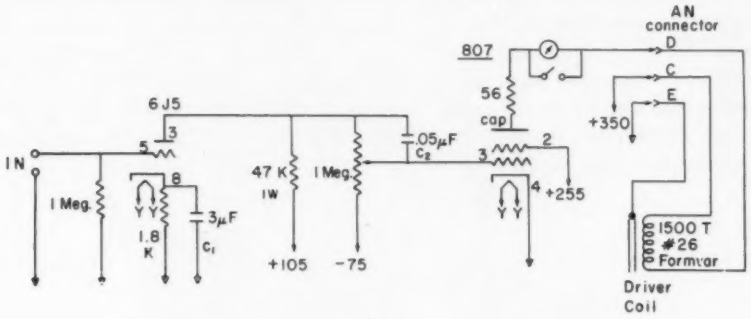
The shape of the diaphragm was dictated by the following considerations: (1) The cross-sectional area presented to the magnetic flux at all points in the magnetic circuit should be at least as large as the cross-sectional area of the core of the coil. (2) The stiffness of the diaphragm should be as small as possible to permit reasonable displacements. (3) The diaphragm should be constrained to travel as nearly like a piston as possible in order to discourage multiple vibration modes which could produce distortion.

All three of these requirements are met by the use of a constant cross-section diaphragm as shown. The diaphragm, cup, and core are made of cold rolled steel. The

transparent chamber is a stock size of glass tubing. The top plate and fittings are stainless steel. The critical dimensions are: air gap, 0.010 inch; core radius, 0.50 inch; free radius of diaphragm 2.0625 inches; diaphragm thickness, 0.25 inch for radius from zero to 0.50 inch. For larger radii, thickness (t) is related to radius (r) by the equation

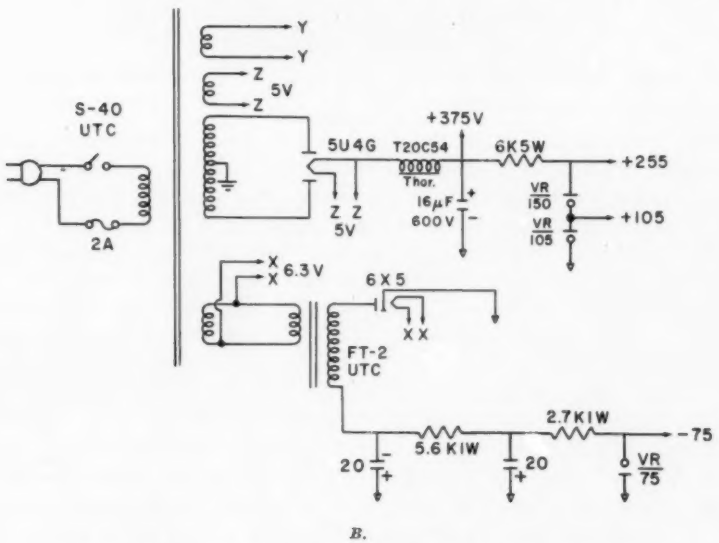
$$t = \frac{1}{8r} \text{(dimensions in inches)}.$$

The electronic circuit is given in Fig. 2*A*. The 6J5 tube is a frequency compensated direct coupled amplifier. The compensation is provided by C_1 and C_2 to correct for the increasing losses in the magnetic driver with increasing frequency. The 807 tube is a direct coupled amplifier tetrode which supplies the required current to the coil. Fig 2*B* is the circuit for the power supply.



A.

Fig. 2*A*.—Schematic diagram of the frequency-compensated amplifier which drives the pressure generator.



B.

Fig. 2*B*.—Schematic diagram of the power supply for the amplifier.

RESULTS

The frequency characteristic of the pressure generator is given in Fig. 3. The compensation provided by the 6J5 circuitry is responsible for the nearly uniform response from d. c. to 100 cycles per second. Although response is shown only to 1,000 cycles per second, the device is usable to slightly above the diaphragm resonance, which occurs at approximately 2,000 cycles per second.

The photograph of Fig. 4 is intended to show constructional details of the pressure generator. The stopcock to the left is a vent for filling the chamber. The monitoring gauge, a mechanically modified Lilly manometer, is at the top left. It is placed with its diaphragm directly exposed to the upper surface of the water in the pressure chamber. Under these conditions, the monitor gauge is resonant at approximately 5,000 cycles per second, so that regardless of its condition of damping its response is essentially uniform to 1,000 cycles per second. The fitting to the right of the monitor gauge accepts the needle or catheter of the test gauge. A rubber grommet, compressed by the knurled thumb screw, grips the needle or catheter and forms a leakproof joint.

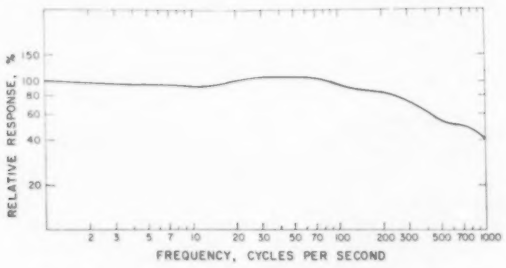


Fig. 3.—Pressure amplitude vs. frequency characteristic of the pressure generator-amplifier combination.

Fig. 5 is a photograph of the complete test setup. The oscillator is at the lower right. The dial of the oscillator is driven by means of a synchronous motor enclosed in the black box. The motor sweeps the oscillator linearly through a decade range of frequency in 90 seconds. Mounted on the same shaft with the oscillator dial is the rotor of a potentiometer. The winding of the potentiometer is energized by a battery, so that the potential between the rotor and one end of the potentiometer increases linearly with shaft rotation and, therefore, with frequency. This voltage connects to the d. c. x-axis input of the oscilloscope, so that the x-position of the oscilloscope spot is linearly related to the frequency of the oscillator. The oscillator output connects to the driver amplifier (shown above the oscillator). The monitor gauge control box is to the left of the oscilloscope. Its output connects to one input of the electronic switch, which is located between the oscilloscope and the oscillator. The test gauge (not shown) connects to the other input of the switch. The switch output connects to the y-axis of the oscilloscope. As the motor drive unit rotates the oscillator tuning shaft, the x-position of the CRT spot moves to the right linearly with frequency and the y-amplitudes of the 2 traces are proportional to the response of the test

and monitor gauges, respectively. If the Polaroid camera shutter is opened at the beginning of the sweep and closed at the end, the developed picture will consist of 2 horizontal bands, the vertical widths of which are proportional to the response of the monitor and test gauges, respectively, and the x-position is proportional to frequency. By this means, a complete frequency response curve over a frequency range of 1 to 1,000 cycles per second may be obtained in a minimum time of about 6 minutes.

Fig. 4.—Photograph of the pressure generator. A description of the fittings and attachments is contained in the text.

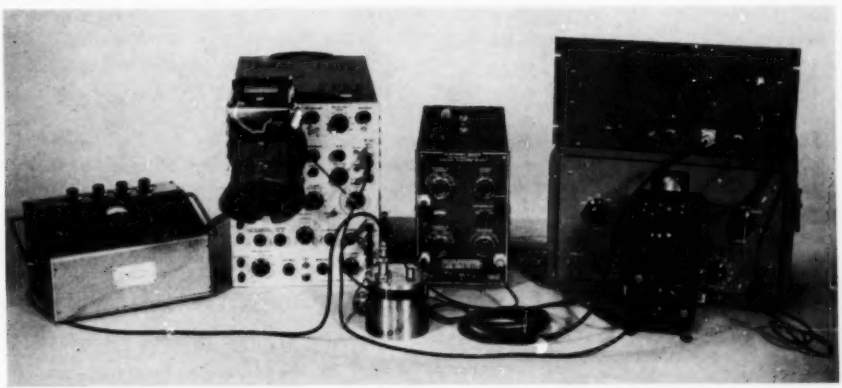


Fig. 5.—Photograph of the complete test setup. Identification of the various components is contained in the text.

DISCUSSION

As a consequence of the good performance of the generator from 0 to 100 cycles per second, it is possible to produce pressure waves of many special shapes by feeding the desired wave into the amplifier unit. Thus by the use of a function generator such as a photoformer,[1] it is possible to check the response of a manometer to pressure waves of the type which the manometer is required to record. For instance, a ventricular pressure curve might be obtained during an open chest operation by direct needle puncture of a ventricle. This curve could then be regenerated in a photoformer and fed through the amplifier to the magnetic driver unit. The ventricular pressure curve would now be reproduced by the pressure generator for an indefinite period of time, and the performance of various manometric systems could be determined. Errors caused by inadequate response of the test manometer could be determined by direct comparison with the monitor waveform.

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THE ENZYMATIC SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF URIC ACID

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THE metabolism of purines is currently attracting increased interest among investigators and clinicians in several areas of clinical medicine. Of fundamental importance to their studies is a reliable method for the determination of uric acid in biologic fluids. Older methods[1-3] which are based on a chemical reaction between uric acid and a chromagen were limited by a lack of specificity and by the need to remove proteins prior to the assay. The latter resulted in a loss of uric acid by coprecipitation. The specificity of these methods was increased markedly by combining the use of the enzyme uricase[4-7] with the colorimetric assay. The enzyme oxidizes uric acid to allantoin, a compound that does not react with the chromagen, and this allows a correction to be made for the presence of substances other than uric acid which do react with the chromagen. Such a modification, however, doubles the number of determinations required for a given sample.

These difficulties were obviated when Kalckar devised an enzymatic spectrophotometric method for the determination of uric acid.[8] No deproteinization is necessary; the assay has an extremely high degree of sensitivity and specificity, and it does not require the routine use of a standard solution of uric acid. In recent years, modifications of the method have been introduced by Praetorius[9, 10] and others,[11] and have been employed in many laboratories, including this one. This paper reports a detailed description of the method, and summarizes experience with more than 4,000 determinations in the course of clinical studies of uric acid metabolism.[12, 13] The accuracy, reproducibility, and limitations of the method have been studied, and additional evidence has been obtained for the extremely high specificity.

METHODS

Principle.—One of the physical properties of the uric acid molecule is its capacity, in aqueous solution, to absorb a definite quantity of light from the ultraviolet region of the spectrum. At pH 9.4 the maximum absorption occurs at 292 mμ with a molar extinction coefficient, ε, of 12,500. The enzyme uricase catalyzes the oxidation of uric acid to allantoin which does not absorb light at 292 mμ. By measuring the optical density of a biological solution at this wave lenth before and after the action of uricase, one can determine the amount of uric acid that it contains. Because of the high degree of purification of available uricase and the marked specificity of this enzyme,[14] the method is similarly specific.

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*These pipettes may be obtained from the A. H. Thomas Co., Philadelphia, Pa., Catalog No. 8204-B, micro sugar pipettes.

Apparatus.—The equipment required for this assay includes: a Beckman spectrophotometer (Model DU) with ultraviolet attachment, silica cuvettes with a 10 mm. light path, micropipettes calibrated to contain 0.1 ml. ± 0.00025 for pipetting serum,* and serologic pipettes of a 0.1 ml. capacity and a graduation interval of 0.001 ml. for pipetting the uricase.

Reagents.—

1. *Uric acid:* Although the routine use of a uric acid standard with each determination is not necessary, a standard solution of uric acid is used to check the method when it is first employed and to assay the activity of uricase preparations. It may also be used to increase the optical density of reference cuvettes (see description of method).

(a) *Purification of commercial uric acid*[15]*: Dissolve 1.1 Gm. of lithium carbonate in 75 ml. of cold distilled water and heat to 90° C. Dissolve 2.5 Gm. of uric acid in this solution, add 0.1 Gm. of activated charcoal, and mix thoroughly. Filter the mixture by gravity into a flask containing 700 ml. of distilled water, preheated to 55 to 60° C., and mix. Precipitate uric acid crystals by the successive addition, at 15 minute intervals, of 2.5 ml., 2.0 ml., and 4.0 ml. of glacial acetic acid. Thirty minutes after the last addition, decant the supernatant liquid and filter off the crystals. Wash the uric acid with distilled water until the filtrate is neutral to litmus paper and dry in a desiccator.

(b) *Standard solutions of uric acid:* Dissolve 30.0 mg. of uric acid in 10 ml. hot water by addition of approximately 1 ml. of a hot, saturated solution of lithium carbonate, and bring the solution to a volume of 100 ml. with distilled water. Such solutions are stable when frozen, but gradually deteriorate at room temperature. A twenty-five-fold dilution (12 micrograms uric acid per milliliter) is used in calibrating the method.

2. *Glycine buffer:* Stock solution two-thirds M, pH 9.4. Dissolve 25 Gm. of glycine and 4.4 Gm. of sodium hydroxide in CO_2-free distilled water and make the final volume 500 ml. Add 3 ml. of chloroform as a preservative. Store in a refrigerator. For use in the assay, dilute the stock solution to 0.1 M for urine determinations and 0.067 M for serum determinations. The dilution can generally be stored at room temperature for a week before microbial growth makes it unsuitable for use.

3. *Enzyme:* A uricase preparation of sufficient purity for use in this method can be prepared from hog liver[16] or obtained commercially.† Commercial preparations were used for the studies reported here. The procedure described below was used for checking the activity, purity, and specificity of uricase. The enzyme in daily use is kept refrigerated and allowed to remain at room temperature only for the brief periods of time required for pipetting enzyme into the cuvettes. Surplus vials of enzyme are stored in the frozen state.

Procedure.—

1. *General description:* The protocol for performing a uric acid determination is given below. The actual analysis is made by determining the change in optical density at a wave length of 292 mμ (referred to as O. D.) which results from uricase action in the assay cuvette. Additional control cuvettes are needed, however, each correcting for changes in optical density resulting from factors other than the enzyme reaction.

(a) *Assay cuvette:* This cuvette contains 2.0 ml. of 0.1 M glycine buffer, pH 9.4, and 1.0 ml. of test solution containing the uric acid to be determined. Uricase is added to this cuvette to catalyze the oxidation of the uric acid. The resulting change in O. D. is determined.

(b) *Enzyme blank cuvette:* The uricase added to the assay cuvette absorbs a small amount of ultraviolet light for which a correction must be made. This cuvette contains 3 ml.

*Uric acid, purchased from the Pfanstiehl Laboratories, Inc., Waukegan, Ill., did not require further purification.

†Purified uricase, obtained from Worthington Biochemical Co., Freehold, N. J., was used in these studies. A purified uricase is also available from Leo Pharmaceutical Products Trading Ltd., Copenhagen, Denmark, and is distributed by Henley & Co., Inc., 202 E. 44th St., New York, N. Y.

of 0.067 M glycine buffer. The change in O. D. resulting from the addition of an amount of uricase equal to that added to the assay cuvette is determined and the value used for such a correction. Only one enzyme blank is determined for a group of assay cuvettes.

(c) *Biological fluid blank cuvette:* Some biological fluids such as serum and spinal fluid contain substances which undergo spontaneous changes in O. D. under the conditions of the assay. The magnitude of such changes must be determined and the appropriate correction applied to the total change in the assay cuvette. This cuvette is a duplicate of the assay cuvette, but no uricase is added.

(d) *Reference cuvette:* This cuvette serves as a reference only and is used for setting the spectrophotometer to zero. The large amounts of nonurate material with absorption at 292 mμ which are present in fluids such as serum can be cancelled out by substituting a reference cell of a corresponding degree of optical density in place of the usual water-containing silica cuvette, thus bringing the initial O. D. reading in the assay cuvette to the range of 0.200 to 0.400. In practice a reference cuvette with an O. D. of 0.850 greater than a silica cuvette is found satisfactory for use in determinations on serum. A reference cuvette of suitable high O. D. may be selected from Pyrex cuvettes, frosted surface of silica cuvettes or the addition of uric acid solution to the silica reference cuvette.*

Solutions are mixed by covering the cuvettes with Parafilm† and inverting them several times. O. D. values are then determined prior to the addition of uricase and are termed *Initial O. D.* Sufficient uricase is added to produce an initial rate of change in O. D. in the assay cuvette of 0.020 to 0.030 per minute. For the commercial preparations of uricase, used by us, 0.005 to 0.010 ml. suffices.

The reaction is allowed to proceed until there is no further change in O. D. in the assay cuvette, usually a period of 25 minutes. O. D. readings are taken at 3, 25, and 30 minutes. The three minute reading is obtained in order to check the activity of the uricase. By this time the change in O. D. should be more than 30 per cent of the total change if the reaction is to be complete by 25 minutes. The thirty minute reading is for the purpose of ascertaining that the reaction is complete. The O. D. at the reaction's end is the *Final O. D.* Initial and final readings are taken for the 2 blank cuvettes, as well as for the assay cuvette.

Calculations.—A solution of uric acid at pH 9.4 containing 1 mcg. per milliliter, will have an O. D. of 0.0745 in a silica cuvette with a 1.0 cm. light path. Oxidation in the presence of uricase abolishes this absorption completely. By determining the change in O. D. in the assay cuvette, resulting specifically from the oxidation of uric acid, the concentration of uric acid can be calculated. The following values must first be available:

(1) △ O. D. assay cuvette = Initial O. D. − Final O. D.
(2) △ O. D. enzyme blank cuvette = Final O. D. − Initial O. D.
(3) △ O. D. biological fluid blank cuvette = Final O. D. − Initial O. D.
(4) △ O. D. uric acid = (1) + (2) + (3).

Then: Quantity of uric acid in assay cuvette in mcg. $= \frac{\triangle \text{ O. D. uric acid}}{0.0745}$ times the total volume of solution in assay cuvette.

By knowing the total amount of uric acid in the assay cuvette and the volume of test solution added to the cuvette in which this amount was contained, one can calculate the concentration of uric acid in the fluid under study.

The initial optical density in the assay cuvette will be reduced slightly as a result of the dilution produced by the addition of uricase. However, in routine assays using up to 0.01 ml. of uricase the magnitude of this reduction in O. D. will be of the order of 1 part in 300, far less than that associated with pipetting errors and for this reason it has been

*A convenient reference cell of high optical density can be devised by placing in an empty silica cuvette a 1 cm. wide strip of perforated metal screen No. 160/40Z obtained as a sample from the Pyramid Screen Corporation, 181 Harvard Street, Brookline 46, Boston, Mass.

†Parafilm, grade M, Marathon Corp., Menasha, Wis.

ignored. The correction assumes significance, however, under conditions where the initial O. D. is extremely high, or the volume of uricase added greatly exceeds that recommended above, and it must then be included in the calculations.

1. *Check of uricase activity:* A formal assay of the activity of each batch of uricase is not generally required, since the 3 minute reading in the routine assay will readily reveal inadequate enzyme activity. However, if difficulties with low activity are encountered, or if the laboratory preparation of uricase is undertaken, a standardized assay of the activity and purity of the enzyme preparation is needed.

In this procedure an assay cuvette (see above) is prepared containing a total of 30 mcg. of uric acid. After the addition of uricase, the solution is mixed and the O. D. at 292 mμ. is read at 1 minute intervals for a total of 3 minutes. An amount of enzyme sufficient to produce a change of 0.100 O. D. unit per minute will be satisfactory for use in the routine determination of uric acid. Smaller amounts can be used, but the time required for completion of the reaction will be correspondingly greater. Usually 0.005 ml. of Worthington commercial uricase contains sufficient enzyme for the determination and will result in a △ O. D. of only 0.001 to 0.010 in the enzyme blank cuvette.

2. *Sample applications of the method:*

(a) *Calibration of the procedure:* A solution of uric acid of known concentration should be analyzed to make certain the procedure is being performed correctly. The procedure is as follows:

(1) Assay cuvette is the same as before except that the 1.0 ml. of test solution contains 12 mcg. of uric acid.

(2) Enzyme blank cuvette is the same as before.

(3) Biological fluid blank cuvette is omitted.

(4) Reference cuvette is a silica cuvette containing water.

Proceed as described previously and then calculate the value of X in the equation below which is the △ O. D. that would have been observed if the concentration of uric acid in the solution, initially contained in the assay cuvette, had been 1 mcg. per ml. Substituting in the equation given above:

$$12 \text{ mcg.} = \frac{\triangle \text{ O. D. uric acid}}{X} \times 3.01^{*}$$

Solve for X. If pure uric acid has been used, and the procedure has been set up correctly, X should equal 0.0745 O. D. unit.

(b) *Assay of urine:* The procedure is as follows:

(1) Assay cuvette is as before, except that the test solution is a dilution of urine. A twenty-four-hour urine collection generally requires a dilution in the range of 1:100 to 3:100 to produce a satisfactory △ O. D. uric acid.

(2) Enzyme blank cuvette is as before.

(3) Biological fluid blank cuvette is unnecessary in routine assay, since changes are usually insignificant. However, such a blank may be required under special circumstances, since urinary metabolites of certain drugs may undergo a spontaneous change in O. D. at 292 mμ. The necessity for use of such a blank may be ascertained by determining the amount of spontaneous change in O. D. occurring in the assay cuvette over a period before addition of uricase. If such changes are significant in magnitude, then a blank will be required.

(4) Reference cuvette—a silica cuvette containing water. The procedure is as previously described and the calculation is as follows:

$$\frac{\text{mg. uric acid}}{\text{per ml. of urine}} = \frac{\triangle \text{ O. D. uric acid}}{0.0745 \text{ O. D. unit/meg./ml.}} \times \frac{3.01^{\dagger} \text{ ml.}}{1 \text{ ml.}} \times \text{dilution} \times \frac{1 \text{ mg.}}{1{,}000 \text{ mcg.}}$$

(c) *Assay of serum:* The procedure is as follows:

(1) Assay cuvette—3.0 ml. of 0.067 M glycine buffer, pH 9.4, and 0.10 ml. of serum.

*Assumes the addition of 0.01 ml. of uricase.
†This example assumes the addition of 0.01 ml. of uricase.

(2) Enzyme blank cuvette—as before. This is read against a silica reference cuvette containing water.

(3) Biologic fluid blank cuvette—same as the assay cuvette but no uricase is added. This blank is of significant magnitude for serum, and it must be determined for each specimen analyzed.

(4) Reference cuvette with a high O. D. is used for reading cuvettes (1) and (2) above. The procedure is as previously described with the following precautions. (1) When initial O. D. readings are taken for serum, note should be made of the slit width reading on the Beckman spectrophotometer and this reading should be reset when the final O. D. is determined. (2) The cuvettes used for serum blank determinations should be free of uricase. It is advisable to set aside several cuvettes to be used only for serum blanks, and never for uricase reactions. (3) It should be noted that the O. D. in the serum blank cuvette may rise or fall, and care must be taken in adding or subtracting this value properly. (4) Since the accurate measurement of the 0.1 ml. of serum is the most critical step of this determination, it is necessary to use micro blood sugar pipettes which are calibrated to contain 0.1 ml. ± 0.00025 of serum. Special care is taken in filling the pipette precisely to the mark, and excess serum is wiped from the tip. The contents are rinsed into the cuvette by drawing buffer solution from the cuvette back into the pipette several times. The calculation is:

$$\frac{\text{mg. of uric acid}}{\text{per 100 ml. of serum}} = \frac{\triangle \text{ O. D. uric acid}}{0.0745 \text{ O. D. unit/mcg./ml.}} \times \frac{3.11^{*}\text{ml.}}{0.1 \text{ ml.}} \times \frac{1 \text{ mg.}}{1{,}000 \text{ mcg.}} \times 100 \text{ ml.}$$

Collection and Preservation of Samples.—

1. *Blood:* For a single determination, a total of 0.3 ml. of serum is required. In practice, approximately 3 ml. of blood was drawn and allowed to clot. No significant difference was found in the urate determinations when serum was removed immediately, as compared to keeping the clotted blood for 8 hours at room temperature, or 24 hours in the refrigerator, prior to removal of serum. Aliquots of separated serum, kept at room temperature for 8 hours, refrigerated for 24 hours, and stored in a freezer for 2 months, showed no significant change in uric acid content. Determined values were the same in serum and plasma and in serum from peripheral venous and arterial blood, nonhemolyzed and slightly hemolyzed samples, and blood drawn with and without stasis.

2. *Urine:* Urine was collected at room temperature because of the tendency of urates to precipitate from chilled solutions. Voidings during a 24 hour period were stored in a bottle containing 4 ml. of toluene as a preservative. Uric acid content was unchanged by storage for 3 days at room temperature. Urate in dilutions of urine prepared for assay was unchanged after 7 days in the refrigerator or after several months at −5° C.

Urate crystals were found to precipitate out of urine samples containing high concentrations of uric acid, even when stored at room temperature. It is possible to assay such samples by redissolving the precipitate in a hot saturated solution of lithium carbonate.

RESULTS AND DISCUSSION

Specificity.—Despite the high degree of specificity of uricase, use of this enzyme to determine uric acid in biologic fluids has many possible sources of error. The specificity of uricase has been proved by testing its reactivity with a large number of pure compounds.[14] However, in urine are many unknown substances which could conceivably react with uricase. Likewise, the uricase solution, although highly purified, still contains a variety of other proteins which may include unknown enzymes. Reaction of such an enzyme with a nonurate substance in urine to produce a substance having either greater or less O. D. at 292 mμ would introduce a corresponding error in the uric acid determination.

*This example assumes the addition of 0.01 ml. of uricase and 0.1 ml. of serum.

An evaluation of the extent of such side reactions is made possible by the fact that the ultraviolet absorption spectrum is a fairly characteristic physical property of uric acid. In order to determine the absorption spectrum of the substance or substances in urine being destroyed by the uricase preparation, the ultraviolet absorption spectrum of urine was determined before and after uricase action and the difference spectrum, corrected for the absorption contributed by the added uricase, was compared with that obtained in a similar experiment with synthetic uric acid. The results shown in Fig. 1 present evidence that the substance in urine destroyed by uricase action has an absorption spectrum indistinguishable from that of uric acid. Similar results were obtained with uricase prepared according to the method of Muller and Bauer.[16] Despite the presence of ultraviolet absorbing substances and uric acid in urine, evidently the uricase preparations used were of sufficient purity that uric acid was the only ultraviolet absorbing material destroyed in significant amounts.

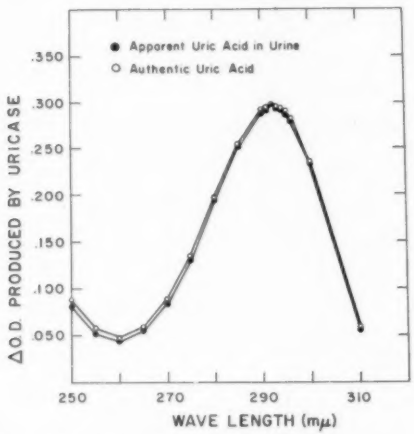
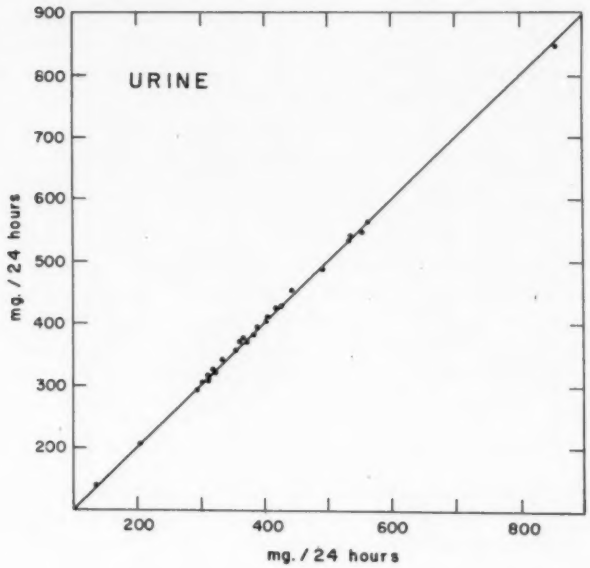


Fig. 1.—A comparison of the absorption spectrum of uric acid with that of the urinary constituent oxidized by uricase. The absorption spectra of urine and of a solution of authentic uric acid in 0.067 M glycine buffer, pH 9.4, were determined. The change in O. D. for each solution resulting from uricase action is plotted above as a function of wave length.

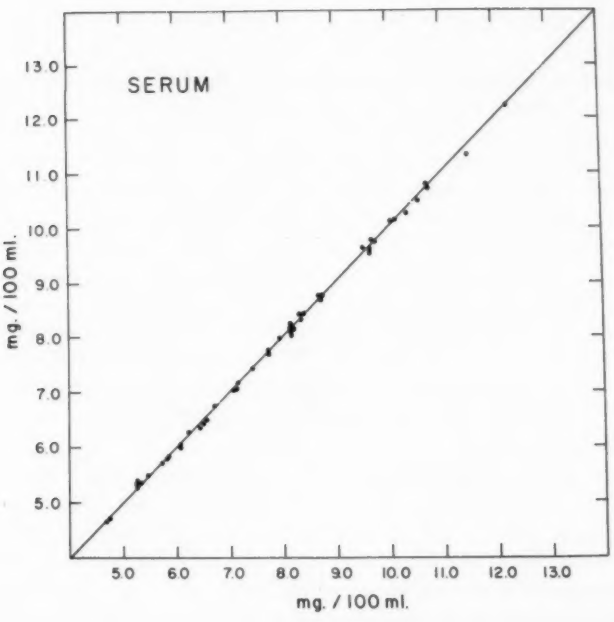
The daily excretion of uric acid by a normal subject on a constant purine-free diet was not altered significantly by the methylated purines present in 6 cups of coffee which were added to the diet. In agreement with the findings of Buchanan and associates,[6] these results indicate that the metabolic products of methylated purines do not interfere in the enzymatic determination as they do in the usual chemical determination.

Reproducibility and Accuracy.—Graphic representations of the reproducibility of uric acid determinations in urine and serum, made by the enzymatic spectrophotometric method, are shown in Fig. 2. The standard deviations* of each group of determinations were ± 3.7 mg. per 24 hours for urine, determined

*Standard deviation[17] = $\frac{2d^2}{2n}$, where d = difference between duplicate determinations and n = number of pairs of determinations.



A.



B.

Fig. 2.—Reproducibility of the enzymatic spectrophotometric method of uric acid determination. The points represent duplicate analyses of the fluids indicated.

TABLE I. RECOVERIES OF URIC ACID ADDED TO URINE AND SERUM

INITIAL URIC ACID CONCENTRATION (MG./100 ML.)	URIC ACID ADDED (MG./100 ML.)	URIC ACID FOUND (MG./100 ML.)	DIFFERENCE (C-A)	RECOVERY (PER CENT)
	3.6	31.7	3.6	100.0
	4.8	32.9	4.8	100.0
	6.0	34.1	6.0	100.0
Urine	9.0	37.2	9.1	101.1
28.1	12.0	40.2	12.1	100.8
	15.0	43.0	14.9	99.3
	30.0	58.2	30.1	100.3
Average recovery				100.2
Range				99.3 - 101.1
	1.20	5.01	1.21	100.8
	2.40	6.22	2.42	100.8
Serum	3.60	7.35	3.55	98.6
3.80	4.80	8.52	4.72	98.3
	7.20	10.91	7.11	98.8
	10.80	14.50	10.70	99.1
Average recovery				99.4
Range				98.3 - 100.8

from 28 paired observations (range 138 to 1,109 mg. per 24 hours), and ± 0.043 mg. per 100 ml. for serum, determined from 50 paired observations (range 4.71 to 12.24 mg. per 100 ml.).

The high accuracy of the method for determining uric acid at a wide range of concentrations is shown in Table I. Known amounts of uric acid were added to aliquots from a sample of urine and a sample of serum, and the recoveries were determined.

The presence of an inhibitor of uricase in a fluid could interfere with the enzymatic spectrophotomatic determination of its uric acid content by preventing the complete oxidation of the uric acid. A variety of compounds can inhibit uricase action when present in appropriate concentrations,[18] but inhibitors would be detected by the assay procedure. The 3 minute reading would indicate a slowing of the reaction rate, similar to the situation that would be observed with an inadequate amount of uricase, and the 25 and 30 minute readings would not be identical, indicating that the reaction had not gone to completion, but was continuing at a slow rate. If inhibition is suspected, at the apparent conclusion of the uricase reaction more uricase should be added to effect complete reaction. In the experience of this laboratory, such inhibition has not been encountered in assaying urine or serum although a very potent inhibitor of uricase, 6 chlorouric acid, has recently been described in the urine of animals receiving 6 chloropurine.[25]

A second potential difficulty with the assay method has been discussed in the description of the biologic fluid blank. Large blanks of this type were encountered in determining uric acid levels of spinal fluid. Had they gone undetected, they would have contributed significant errors to the determinations. Recently, Praetorius[19] has presented evidence to show that the interfering substance in spinal fluid is ascorbic acid, and he presents a procedure by which the difficulties due to this compound may be circumvented. Since it is possible that under certain conditions, for example in its therapeutic administration,

substantial amounts of ascorbic acid may be excreted in the urine of patients, we gave two 1 Gm. doses of this compound, in a 12 hour period, to a normal subject who was eating a constant diet that was poor in purines. The biologic fluid blank during the day of administration rose from undetectable values to a value that was 6 per cent of the △ O. D. of the assay cuvette for urine.

Urine from alcaptonuric patients exhibits a biologic fluid blank from spontaneous oxidation of homogentisic acid that is so great as to preclude the use of the spectrophotometric assay method as described here. However, by using phosphate buffer at pH 7.4, in place of glycine buffer at pH 9.4, and with larger quantities of uricase, it was possible to reduce the value of the biological fluid blank to very low levels, thereby permitting the determination to be made.

It is apparent, then, that when analyzing fluids from sources not routinely studied, or from patients receiving large quantities of a drug, it is advisable to determine whether a biologic fluid blank occurs and whether it is feasible to correct for this blank.

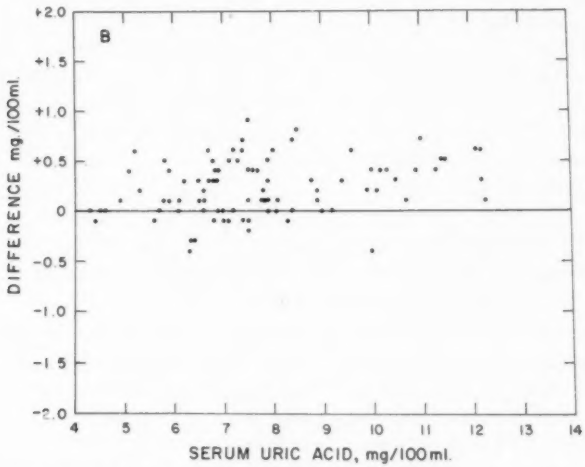


Fig. 3.—Comparisons of values for serum uric acid as determined by the enzymatic spectrophotometric method and colorimetric methods. The abscissa indicates the values for serum uric acid as determined by the enzymatic spectrophotometric method. The ordinate indicates the difference between this value and the colorimetric method.

Comparisons with Other Methods.—The method described in this paper includes minor modifications of the procedure originally described by Praetorius for the determination of uric acid in serum. Introduction of the determination of the enzyme blank eliminates the need to plot graphically the decline in optical density accompanying uricase action.[9] It also eliminates a small but recurrent error in determining initial O. D. which was due to the occurrence of a brief lag period in the reaction following the addition of uricase.

In contrast to the enzymatic spectrophotometric method, many of the colorimetric procedures for the determination of uric acid include a deproteinization step. When determined by the former method, the recovery of uric acid added to a sample of serum was 99.4 per cent before, and 92 per cent after

deproteinization of the serum by treatment with tungstic acid.[2] This potential error of approximately 7 per cent is obviated by use of the enzymatic spectrophotometric method since deproteinization is not required.

During the course of the studies in this laboratory, the Chemistry Laboratory of the Clinical Pathology Department of the Clinical Center of the National Institutes of Health employed a colorimetric method for the assay of uric acid. Simultaneous determinations of serum uric acid were performed in the 2 laboratories at intervals during the past year and comparisons of the results obtained by use of the 2 methods are presented in Fig. 3. The colorimetric method of Stern and Stransky,[3] as modified by Archibald,[20] was used. It is reported to avoid coprecipitation of uric acid and proteins.[21] Although a comparison of other procedures for the determination of uric acid gave divergent results,[22] the Archibald modification resulted in values which were in good agreement with those obtained by the enzymatic spectrophotometric method, with the majority of values agreeing within 0.5 mg. per 100 ml. The determinations by the enzymatic spectrophotometric method average 0.3 mg. per 100 ml. higher than determinations by the Archibald procedure.

Despite this excellent agreement between the colorimetric method and the enzymatic method for determination of uric acid in serum, the lack of specificity of the colorimetric procedure can lead to erroneously high values under certain circumstances. Previous workers have demonstrated that administration of salicylates results in erroneously high values for urinary uric acid when determined by the colorimetric procedure[23] due to the presence of gentisic acid.

We have found the presence of high levels of salicylate in the serum to be associated at times with falsely high values for serum urate. Determinations by the colorimetric procedure were as much as 75 per cent higher than those made by the enzymatic spectrophotometric method.[24]

Comment.—The determination of uric acid by use of the enzymatic spectrophotometric method of Kalckar[8] and Praetorius[9] has an accuracy of better than 2 per cent as used in this laboratory with either urine or serum. It is highly specific and is consistently reliable regardless of the presence of salicylates or metabolites of methylated purines. It is a reproducible microanalytical method that is quite simple in theory and application. Since it depends on an intrinsic property of the uric acid molecule, the routine use of a standard solution of uric acid is obviated. The error introduced by protein precipitation in other methods is eliminated by the omission of this step in the present method.

The details involved in a practical application of the method are no deterrent to its use. Most clinical laboratories are equipped with a Beckman spectrophotometer. The time required for the assay is reasonable. The enzyme, uricase, is commercially available in a satisfactory state of purification and is stable if properly stored. Samples of urine and serum may also be stored for reasonable periods of time prior to assay.

SUMMARY

A detailed description of the procedure used in this laboratory for the enzymatic spectrophotometric determination of uric acid in biologic fluids is

presented. Studies of its accuracy and reproducibility as well as further evidence for its high degree of specificity have been included.

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PROCEEDINGS OF THE CENTRAL SOCIETY FOR CLINICAL RESEARCH

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ABSTRACTS

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79. THE SIGNIFICANCE OF AMINO ACID TRANSPORT IN PROTEIN SYNTHESIS

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It has been suggested that the process by which amino acids are transported intracellularly and concentrated may function as a regulator of protein synthesis. The concentrating mechanism of various tissues in the rat has been studied in three conditions associated with marked alterations in protein metabolism—aging, hypopituitarism, and hypothyroidism.

The nonutilizable amino acid α-aminoisobutyric acid-1C^{14} (AIB) was used to examine the cellular penetration of amino acids independent of other metabolic transformations in which they participate. The steady-state concentration gradient (intracellular/plasma concentration), taken as a measure of concentrating ability (amino acid transport), was determined for liver, heart, gastrocnemius, diaphragm, and pituitary under standardized conditions. These consisted of injecting intravenously 0.55 mg. per 100 gram body weight in nephrectomized animals (nephrectomized to prevent urinary loss and thereby sustain higher plasma levels), or injecting intraperitoneally 0.1 mg. AIB per 100 gram body weight in unoperated animals. Plasma and tissue samples were obtained 24 hours later when a steady state had been reached.

Increasing age (40 days versus 125 days) was associated with a striking *decrease* in the concentrating ability of heart (–76 per cent), gastrocnemius (–55 per cent), diaphragm (–63 per cent), and pituitary (–30 per cent), and a marked *increase* in liver (+46 per cent). Hypophysectomy produced similar changes of lesser magnitude—heart (–10 per cent), gastrocnemius (–27 per cent), diaphragm (–15 per cent), and liver (+33 per cent).

The effect of thyroidectomy, evident within thirty days, was similar to that observed with hypophysectomy and increasing age and was more marked in the younger animal (77 days versus 125 days)—heart (–44 per cent, –14 per cent), gastrocnemius (–40 per cent, –29 per cent), diaphragm (–26 per cent, –5 per cent), and liver (+61 per cent, +45 per cent). Pituitary weight, both absolute and relative to body weight, increased appreciably (+29 per cent, +65 per cent) in the thyroidectomized animal and was associated with a marked increase in amino acid concentrating ability (+120 per cent).

It is concluded that the amino acid transport process is responsive to physiologic conditions affecting protein metabolism. A striking dissociation of liver and peripheral tissue activity is evident in respect to amino acid transport. Since decreased growth is common to all three states studied, it is suggested that these results reflect in part either a decrease in growth hormone level (hypothyroidism, hypopituitarism) or a decreased tissue responsiveness (aging).

80. SALT LOADING AND EXCHANGEABLE ELECTROLYTES IN PATIENTS WITHOUT AND WITH MILD HYPERTENSION

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We reported previously (J. Lab. & Clin. Med. 50: 938, 1957) electrolyte information on a control group and 12 patients with mild hypertension. We extended this study to define transitional changes in renal function and electrolyte metabolism between a control group of 20 young male patients (< 50 years of age, mean blood pressure [MBP] 97 mm. Hg) without serious illness or evidence of cardiovascular or renal disease and 31 male patients of comparable age with mild essential arterial hypertension. The hypertensive patients were separated by blood pressure levels into low ($n = 16$, MBP 107) and high ($n = 15$, MBP 130) groups. We measured clearance of inulin (C_I), PAH (C_{PAH}), sodium (C_{Na}), potassium (C_K), and chloride (C_{Cl}) before and after a salt and water load. Exchangeable sodium (E_{Na}), potassium (E_K), and bromide (K_{Br}) were measured on a different day.

The surface area of the hypertensive groups was greater than the control, and body fat, estimated from an empiric formula, was also larger in the hypertensive groups ($p < 0.01$). C_I was the same in all groups; mean C_{PAH} was 660 ± 138 ml. per minute per 1.73 M^2 in the controls, 635 ± 154 in the low, and 594 ± 111 in the high group, but the differences were not significant. There was no difference in serum sodium or in sodium excretion prior to loading. After the salt challenge, all groups showed a rise in absolute sodium excretion (control, 84 ± 54; low, 95 ± 49; high, 146 ± 140 μEq per minute) of insignificant difference.

E_{Na} in the control, low, and high groups was 41.6 ± 4.0, 40.9 ± 8.8, and 39.7 ± 5.1 mEq. per kilogram, respectively; E_K, 45.6 ± 5.0, 44.8 ± 8.5, 40.0 ± 4.6; E_{Br}, 28.7 ± 2.5, 28.3 ± 4.1, 26.5 ± 2.2.

These data indicate that the electrolyte and renal abnormalities of patients with severe hypertension are not seen in those patients with mild disease. Trends, not all statistically significant, can be seen between these groups which suggest that these derangements are secondary to the ravages of the disease and the fatness of the patients. They offer no support for the hypothesis that sodium metabolism is altered initially in essential arterial hypertension.

81. ERYTHROPOIETIC ACTIVITY OF PLASMA IN POLYCYTHEMIA

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The presence of a factor in whole plasma that enhances the erythrocyte utilization of radioiron (Fe^{59}) in the rat has been evaluated in 50 subjects with polycythemia. Whole plasma injected as 3 per cent body weight subcutaneously in the starved rat influences the turnover of tracer iron depending on the source of the plasma. Each test plasma is run in 5 rats and each experiment is controlled by a normal plasma group and a known active plasma group.

The mean activity of plasma from patients with both polycythemia vera and secondary polycythemia is greater than that of normal subjects and generally less than known active plasma from anemic subjects. Plasma of polycythemia subjects whose hemoglobin is greater than 17.0 Gm. per 100 ml. blood has more activity than that below 17.0 due to phlebotomy or prior therapy. Animals given erythrocytes to make them polycythemic have a depressed iron turnover and erythropoietic activity of serum but retain the ability to respond to added serum of bled rabbits. This activity can be demonstrated in starved, hypophysectomized, thyroidectomized, and polycythemic rats. A female patient with hypernephroma and polycythemia has been studied. Plasma from preoperative phlebotomies showed activity comparable to that of the known active control while the postoperative plasma was the same as the normal human control. A whole saline extract of the tumor was high in erythropoietic activity. Extracts of hypernephroma from a second patient without associated polycythemia showed no activity beyond that of normal human control plasma. A third patient with polycythemia associated with brain tumor had highly active plasma.

These experiments indicate that either primary polycythemia or polycythemia secondary to hypoxia or associated with hypernephroma is correlated with a plasma factor that enhances the turnover of radioiron. The more severe degree of polycythemia seems correlated with the erythropoietic activity of the plasma. Following removal of a renal-cell carcinoma associated with polycythemia, erythropoietin activity decreased and the tumor extract was as active as the preoperative plasma.

82. EVIDENCE FOR A DIRECT RENAL TUBULAR ACTION OF PARATHYROID EXTRACT

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Fasting, hydropenic mongrel dogs, anesthetized with pentobarbital sodium, were prepared by catheterizing each ureter for separate urine collections and inserting a polyvinyl cannula transaortically into the right renal artery. A constant-speed (1.27 ml. per minute) infusion of normal saline with added heparin was employed to keep the arterial cannula patent. A constant-speed (3.0 ml. per minute) intravenous infusion of normal saline containing appropriate amounts of inulin and para-aminohippurate was administered during each experiment. After a suitable number of control periods of 20 to 30 minutes each, during which glomerular filtration rate and effective renal plasma flow were approximately the same in the "experimental" and "control" kidney, purified parathyroid extract prepared by one of us (H. R.) was added to the

†Deceased.

renal arterial infusate. Dosage rates of 0.33 gamma per minute or less were employed. In a few experiments, commercial parathyroid extract (Lilly) was used. Observations on differential renal functions were then continued for 3 to 4 hours, utilizing clearance periods of 15 to 45 minutes each.

Phosphate excretion increased within 30 minutes after starting parathyroid extract. This effect was either restricted to or greater in the experimental kidney than in the control kidney. The phosphaturia was not accountable for by changes in glomerular filtration rate or concentration of plasma phosphate, a decrease occurring in both the absolute rate of phosphate reabsorption and the fraction of filtered phosphate reabsorbed.

These data support the following conclusions: (1) Parathyroid extract increases the excretion of phosphorus by a direct action on the kidney, rather than indirectly through hemodynamic, secondary humoral, or extrarenal changes. (2) This effect is primarily tubular in origin. (3) Recirculation of the hormone may occur since similar (but lesser) effects were frequently noted to occur in the contralateral kidney.

83. NONPERISTALTIC ESOPHAGEAL MOTILITY ACCOMPANYING EXPERIMENTALLY PRODUCED HEARTBURN

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Intraesophageal infusion of N/10 HCl has been suggested by several groups as being a reliable test for esophagitis. Acid-pepsin reflux, detected by lowering of intraesophageal pH, was considered the causative mechanism in the production of heartburn. A study was undertaken to assess the influence of distention, temperature extremes, and pH on the production of heartburn, utilizing a protocol modified from the method of Jones (1938). Intraesophageal pressure tracings were obtained with three tandem fluid-filled, open-tipped catheters at 5 cm. intervals, connected externally to Statham P23D strain gauges and a Sanborn multichannel recorder. The lowermost catheter was positioned at the diaphragmatic hiatus, and fluids were infused intraesophageally through a fourth tube whose tip was located at the level of the middle catheter. Continuous infusion of normal saline and N/10 HCl was performed according to the method of Bernstein.

The 34 subjects included normal controls, patients with hiatal hernia, achalasia, peptic ulcer, functional bowel disorders, postgastrectomy syndrome, and total gastrectomy. Twenty-one subjects had a pre-existing history of heartburn. Only those with clinical indications of anatomic esophageal disease were subjected to esophagoscopy.

Analysis of the results revealed that the continuous N/10 HCl infusion represented the most effective method of producing heartburn. Abnormal nonperistaltic motor activity, i.e., rhythmic esophageal pressure waves in the absence of a normal deglutition pressure gradient across the three recording sites, was observed in 18 of 19 instances in which heartburn was produced synchronous with the duration of the heartburn. Less effective agents for the production of heartburn, in decreasing order, were: continuous infusion of normal saline, 10 ml. of N/10 HCl, and rapid 30 ml. infusion of warm water (42° to 44° C.). Concurrent disordered motility was demonstrated equally consistently when heartburn resulted. Nonperistaltic motor activity followed each of 9 different infusions in one patient who had severe heartburn following a total gastrectomy. Basal nonperistaltic activity was observed in patients with hiatal hernia and peptic ulcer, spontaneously, suggesting the presence of reflux.

It is concluded that heartburn is a nonspecific symptom associated with abnormal nonperistaltic esophageal motility induced principally by stimulation by acid or by distention.

84. REVERSAL OF THE DIURNAL URINARY EXCRETION OF WATER AND ELECTROLYTES AS A MANIFESTATION OF PRIMARY HYPERALDOSTERONISM

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Nocturnal polyuria of 6 years' duration led to the discovery of primary hyperaldosteronism (adrenal tumor) in a schizophrenic patient who had been hospitalized for 25 years. Review of hospital records suggested that hyperaldosteronism was present for about 12 years before study.

A remarkable reversal of the normal diurnal pattern of urinary water and electrolyte excretion was found. With normal activity and a general diet, the night specimens (8 P.M. to 8 A.M.) contained over 80 per cent of the daily urinary water, sodium, chloride, potassium, and total solutes. The urinary sodium/potassium ratio was 0.56 in the day and 2.89 in the night specimens. The patient did not voluntarily ingest water during the night. When kept at bed rest, urinary water and electrolytes were nearly equal during each 12-hour period. Postural hypotension was not demonstrated.

Postural alteration of the diuresis induced by a liter of water was qualitatively normal but quantitatively exaggerated (complete excretion in one hour if supine; about ⅓ of the load excreted in 4 hours when standing). The antidiuresis of quiet standing was somewhat diminished by leg wrapping and greatly decreased by alcohol ingestion. Other evidence indicated normal osmoreceptor and exaggerated volume-receptor control of ADH release. The renal tubules were responsive to vasopressin. Posture so altered renal sodium excretion that a 9.0 Gm. NaCl load (3 per cent saline) was completely excreted in 90 minutes while supine, but only about 50 per cent eliminated in 140 minutes while tilted head-up to 20°. Peak saluresis was about 2,600 μEq per minute when supine and 1,400 μEq per minute when tilted. Mean creatinine clearances were identical in these experiments.

These and other data suggest that the renal mechanisms for excretion or conservation of salt and water were unusually sensitive to changes in the effective blood volume. Some unidentified factor seemed to oppose aldosterone, preventing excessive volume expansion. The normal "escape" from sodium retention during aldosterone administration and "paradoxical" saluresis by patients with primary hyperaldosteronism given ACTH may be related to these findings. It is felt that this phenomenon may account for the nocturnal polyuria incidentally noted in other reported cases of primary hyperaldosteronism.

85. THE CONFUSION OF DISTRIBUTION AND DIFFUSION

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The available methods for measuring pulmonary diffusing capacity for carbon monoxide (D_{LCO}) assume that the lungs are made up of units having a uniform ratio of ventilation or volume to diffusing capacity. Fowler, Cornish, and Kety (J. Clin. Invest. 31: 40, 1952) have established that the lungs, in most cases, are not evenly ventilated. We have combined the nitrogen washout

method of Fowler and associates with the determination of steady state D_{LCO} as suggested by Forster (J. Appl. Physiol. 11: 277, 1957) to study the effect of the distribution of inspired gas on D_{LCO}. The extent of uneven ventilation and the steady state D_{LCO} were measured by breathing a helium-oxygen-carbon monoxide mixture during a control period and after the inhalation of a Mecholyl-histamine aerosol. In 3 subjects the aerosol increased the extent of uneven ventilation (N_2 clearance delay rose from 14, 27.5, and 60.8 per cent to 27.8, 163.2, and 100 per cent, respectively) while steady state D_{LCO} fell from 21.2, 21.3, and 24.2 ml./mm. Hg × min. in the control period to 12.7, 9.8, and 16.1 ml./mm. Hg × min. after the aerosol. In contrast, D_{LCO} measured by the rebreathing method developed in this laboratory, when performed immediately after completion of the steady state studies, rose somewhat (26.2, 23.4, and 37.5 ml./mm. Hg × min. to 27.8, 31.4, and 44.0 ml. mm. Hg × min.), probably because of the increased work of breathing (oxygen consumption rose from 353 and 423 ml./min. to 712 and 639 ml./min.) under conditions of bronchospasm. In 2 subjects, Mecholyl-histamine aerosol did not increase N_2 clearance delay nor alter steady state D_{LCO} significantly. These studies support the theoretical predictions that the D_{LCO} values obtained by the steady state method are influenced by the extent of uneven ventilation while the rebreathing method more nearly measures the true D_{LCO}.

86. EFFECT OF ETHANOL ON FATTY ACID METABOLISM IN VITRO

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Because of the known clinical relation between alcohol and fatty liver, the effect of ethanol on fatty acid synthesis was studied in rats in vitro.

Incubation of liver slices with ethanol-C^{14} or acetate-C^{14} at 0.5 mM concentration resulted in similar labeling of fatty acids, indicating that ethanol per se is not a better precursor for fatty acid formation than acetate. However, at 20 times higher substrate concentrations (10 mM) incorporation of the label into fatty acid with ethanol was on the average 6.9 times greater than with acetate, suggesting that ethanol metabolism may stimulate fatty acid synthesis. This was confirmed by incubating liver slices with a small amount of acetate-C^{14} (0.33 mM) together with either 10 mM unlabeled ethanol, 10 mM unlabeled acetate or 5 mM unlabeled glucose. In each of the 10 animals studied, incorporation of acetate-C^{14} into fatty acids was highest in the presence of unlabeled ethanol. On the average, labeling of the fatty acids with ethanol was 6.5 times greater than with acetate and 2.9 times greater than with glucose.

The mechanism of this effect is still unknown: in liver slices incubated with similar concentrations of ethanol, oxygen uptake and palmitate-C^{14} oxidations were not altered, nor did alcohol appear to increase the activity of the hexose monophosphate shunt, as determined by $C^{14}O_2$ production from glucose-1-C^{14} and glucose-6-C^{14}. In adipose tissue (epididymal fat pad), oxidation of ethanol was much less than in liver slices, and ethanol did not enhance fatty acid synthesis from acetate-C^{14}. This indicates that oxidation of ethanol is probably essential for the stimulatory effect on fatty acid formation in liver slices.

Since in the liver, oxidation of ethanol is coupled with reduction of diphosphopyridine nucleotide and since in vitro reduced diphosphopyridine nucleotide increases fatty acid formation, it is suggested that in liver slices the observed stimulatory effect of ethanol on fatty acid synthesis is related to the reduced phosphopyridine nucleotide formed as a result of the oxidation of alcohol.

87. THE IN VITRO THYROID RESPONSE TO PROPYLTHIOURACIL

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(INTRODUCED BY ROBERT D. LANGE, M.D.)

Ability to concentrate iodine has been demonstrated by minced beef thyroid tissue incubated for 2 hours in Krebs-Ringer-phosphate buffer containing radioactive iodine. Supplementation with 1 to 20 mu./ml. (milliunits per milliliter) of thyrotropin results in a proportional increase in radioiodine uptake. Although thyroid glands differ widely in their ability to concentrate I^{131}, reproducibility of iodine uptake of a given thyroid mince preparation is high. Application of the techniques of paper chromatography and radioautography to hydrolyzed thyroid mince following incubation demonstrates radioactivity in areas corresponding to monoiodotyrosine, diiodotyrosine, and iodide. In addition, thyroxine and triiodothyronine are observed proximal to or superimposed on unknown radioiodinated compounds near the solvent front. Monoiodotyrosine-I^{131} and diiodotyrosine-I^{131} are increased with the addition of thyrotropin prior to incubation. Iodide and solvent front radioactivity both decrease with thyrotropin.

The effect of n-propylthiouracil on thyroid tissue was studied with the technique described above. In medium containing 10^{-3} M. n-propylthiouracil, iodine uptake was reduced from 43.6 ± 7.9 per cent to 23.3 ± 3.6 per cent. A similar trend was observed when the n-propylthiouracil containing medium was supplemented with 20 mu./ml. thyrotropin. Uptake decreased from 61.6 ± 6.9 per cent to 31 ± 4.9 per cent. In the levels of n-propylthiouracil from 10^{-3} to 10^{-7} M., the addition of thyrotropin always resulted in some increase of the I^{131} uptake. The formation of monoiodotyrosine-I^{131} and diiodotyrosine-I^{131} was negligible with 10^{-3} M. n-propylthiouracil. As the concentration of n-propylthiouracil decreased, the level of iodotyrosines approached the control value. Propylthiouracil induced a proportionately greater inhibition of the formation of diiodotyrosine-I^{131} than of monoiodotyrosine-I^{131}. Radioactivity at the solvent front was markedly increased with n-propylthiouracil. In contrast with the control preparation, a further increase in solvent front radioactivity occurred with the addition of thyrotropin.

According to classical theory, the formation of diiodotyrosine and its subsequent coupling is a necessary prerequisite to thyroxine synthesis. The present studies indicate that levels of n-propylthiouracil sufficient to depress diiodotyrosine formation allow a significant I^{131} uptake with the formation of limited amounts of monoiodotyrosine and perhaps other iodinated amino acids.

88. EVIDENCE FOR A DIRECT AND IMMEDIATE EFFECT OF INSULIN ON HEPATIC GLUCOSE OUTPUT IN DIABETIC AND NORMAL DOGS

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(INTRODUCED BY MARVIN D. SIPERSTEIN, M.D.)

The role of insulin in hepatic glucose metabolism remains an unresolved controversy. In these studies the effect of insulin on net glucose balance across the liver was measured in diabetic and nondiabetic dogs with portacaval shunts; this preparation permits the measurement of *hepatic* rather than splanchnic

glucose metabolism. Hepatic blood flow was measured in 19 experiments by the technique of Bradley utilizing I^{131} rose bengal as the extractable material. Arterial and hepatic venous blood was sampled at 10-minute intervals, during the 40-minute control period and the 60 minutes' duration of insulin infusion. Blood glucose was analyzed in triplicate by Somogyi's titrametric method.

In diabetic dogs insulin produced an immediate drop in hepatic glucose output which fell 51 per cent from 77 to 38 mg. per minute. A decline of similar magnitude (44 per cent) in hepatic glucose output from 45 to 25 mg. per minute occurred in the nondiabetic dogs during insulin infusion.

In addition, the effects on hepatic glucose output of a glucose load intravenously infused over 90 minutes were compared in diabetic dogs before and after insulinization. In 3 mildly diabetic dogs (mean control blood glucose 145 mg. per cent), the glucose infusion produced an uptake of glucose by the liver when mean arterial glucose concentration reached 195 mg. per cent (range 168-209 mg. per cent). By contrast, in 3 more severely diabetic dogs (mean control blood glucose 238 mg. per cent), glucose storage by the liver did not occur despite a rise in arterial glucose concentration to levels between 350 and 490 mg. per cent. When, however, these same severely diabetic dogs were pretreated with insulin immediately before glucose infusion, hepatic glucose *uptake* (negative net balance across the liver) occurred at a mean arterial glucose concentration of only 158 mg. per cent (range 140-168 mg. per cent).

These data indicate that insulin has an immediate and direct effect on hepatic glucose output both in normal and in diabetic dogs. Moreover, not only is the arterial glucose level at which the liver stops contributing glucose and starts retaining glucose elevated in diabetic dogs, but this level is promptly lowered toward normal by insulinization.

89. THE EFFECT OF INSULIN ON THE HEPATIC OUTPUT OF GLUCOSE IN THE DOG

F. I. R. Martin, M.D. (By Invitation), Jack R. Leonards, M.D. (By Invitation), James W. Craig, M.D., Frank Barry, M.D. (By Invitation), Max Miller, M.D., J. Ashmore, M.D. (By Invitation), and W. Shoemaker, M.D. (By Invitation), Cleveland, Ohio

The concept that one of the actions of insulin in the intact animal is to lower significantly the output of glucose from the liver is at present highly controversial. Experiments so far reported by various workers have been conflicting in their conclusions and are difficult to compare because of the different techniques employed. In an attempt to resolve this situation experiments have been carried out in unanesthetized dogs in which chronic indwelling catheters were maintained in a hepatic vein, the portal vein, and the aorta. Hepatic glucose output was determined by direct measurement of the difference in plasma glucose concentration across the liver and the estimated hepatic plasma flow and also by consideration of the difference in specific activity of glucose-C_{14} in the hepatic and portal veins. An independent determination of the hepatic glucose output was also obtained indirectly from the specific activity-time curve of glucose-C_{14} in arterial samples drawn at the same time.

The results obtained demonstrate that when small doses of insulin (0.2 unit per kilogram in 1 hour) are given intraportally to unanesthetized dogs, there is no significant decrease in over-all hepatic glucose output, while the peripheral utilization of glucose is greatly increased. In all experiments there was good agreement among the various methods used, except that the results of the indirect glucose-C_{14} method were consistent qualitatively but not quantitatively. A few additional experiments in which larger doses of insulin were

given rapidly intravenously were confirmatory of those described above. It is concluded that the hypoglycemia produced by insulin in the intact dog, whether given slowly intraportally in small doses or intravenously, is not caused by a reduction in the output of glucose by the liver.

90. POTENTIAL RECOVERY OF THE ATROPHIC KIDNEY IN EXPERIMENTAL UNILATERAL RENAL ARTERY STENOSIS

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(Introduced by I. H. Page, M.D.)

The most common surgical treatment in unilateral renal artery disease is uninephrectomy, an unnecessarily wasteful procedure when an atrophic kidney still retains some function. Therefore, the more conservative approach which consists in reconstructive surgery of the renal artery has been advocated. The success of the last procedure will depend in part on the ability of the affected kidney to regain fully its functions. Potential renal recovery was explored in the present experiments. Left renal atrophy was elicited in rats by partial constriction of the aorta with silver clips placed between the origins of the 2 renal arteries. Left uninephrectomy and/or unclamping were performed at the same time as aortic constriction, or 20 or 40 days later. Results were expressed by the kidney ratio: left kidney weight/right kidney weight, which normally is equal to 1. Other criteria were survival of the animals, blood pressure determinations, and heart weights.

After 20 and 40 days of aortic stenosis, kidney ratios were 0.76 and 0.68, respectively. When right nephrectomy was performed at the same time as aortic stenosis, there was no increase in mortality and left renal atrophy was prevented as shown by a kidney ratio greater than 1. Unclamping 20 or 40 days after stenosis did not alter the respective weights of the 2 kidneys (kidney ratio values of 0.76 and 0.80, respectively); however, when associated with right nephrectomy, the left kidney hypertrophied (kidney ratio values between 0.9 and 1.16). The same degree of hypertrophy (kidney ratio 0.99) took place when the clamp was left in place at the time of right nephrectomy. Hypertension was remitted more regularly by left nephrectomy than by unclamping, as evidenced from blood pressure determinations and heart weights.

It is concluded than an atrophic kidney can hypertrophy and recover functionally as long as there are no degenerative lesions, in spite of reduction in renal blood supply; such a kidney remains responsive to renotrophic stimuli.

91. ELEVATIONS OF SERUM CHOLESTEROL IN MAN IN ASSOCIATION WITH LIFE STRESS AND INDEPENDENT OF DIET AND EXERCISE

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In an earlier study of ambulatory patients with indications of coronary artery disease, evidence was adduced that meaningful life situations interpreted as burdensome by the patient may evoke a significant rise in the serum concentration of cholesterol. Because of the potential importance of this observation and in order to provide more rigorously controlled data, the present study was undertaken with 3 similar patients, hospitalized for periods of from 4 weeks to nearly 6 months on a metabolic ward with diet and exercise maintained constant. The constant diets were "average American" with approximately 40

per cent of the caloric content in fat. Psychological data were gathered through daily interviews, repeated psychological testing, and direct observation of behavior on the ward. Judgments made as to the degree of prevailing emotional stress were recorded and kept scrupulously separate from chemical determinations of serum cholesterol made two or more times a week. Such long-term observations yielded elevations in serum cholesterol of 20 to 35 per cent (as much as 75 mg. per cent) occurring despite the constant diet and exercise. The elevations coincided with the separately judged stress periods at a better than 1 per cent level of confidence. One of the subjects died of recurrent coronary occlusion while on the metabolic ward and during a period of great personal turmoil. Similarly in short-term stress interviews, significant elevations of serum cholesterol as great as 35 mg. per cent were induced within one hour. The observations were compared blindly with "neutral" interviews and the difference was significant also at the 1 per cent level of confidence. It is concluded that meaningful situations are capable of participating in the regulation of the serum concentration of cholesterol independent of diet and exercise. Furthermore, changes under circumstances of stress may occur within an hour.

92. THE EFFECTS OF SEROTONIN ON SEGMENTAL CORONARY VASCULAR RESISTANCE

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(INTRODUCED BY STUYVESANT BUTLER, M.D.)

Recent interest in amine oxidase inhibitors in coronary artery disease has attributed a possible coronary vasodilator role to serotonin. This study was undertaken to determine precisely the effect of serotonin on the resistance in different segments of the coronary tree without the conflicting influence of variations in extravascular coronary support from changes in cardiac work.

The heart was exposed in the anesthetized dog through a left thoracotomy. The left main coronary was isolated, cannulated with a Gregg cannula and perfused with arterial blood from a donor dog. The experimental animal was exsanguinated rapidly, and its heart, with left coronary flow maintained by perfusion, was excised and transferred to a gauze sling. The perfusion was kept at a known and constant rate by a blood pump interposed between the heart and donor dog. Oxygen content and temperature of the blood were kept constant. Pressures were recorded simultaneously in the left coronary artery, a small (1.0 mm. diameter) coronary artery, and a small coronary vein through indwelling polyethylene catheters. The coronary sinus drained to the exterior at atmospheric pressure. Knowing coronary flow, it was possible to calculate resistance at the sites noted. The perfused isolated heart beat slowly, performed no external work, and thus eliminated intraventricular pressure change as an extravascular factor in varying coronary resistance.

Serotonin was infused into the left main coronary in a dose range from 10 to 100 mcg. per minute. Following infusion there was a consistent fall in mean pressure in the large and small artery segments with either no change or a fall in small vein pressure. Total calculated coronary resistance fell with the major component occurring in the segment between small coronary artery and vein. Resistance changes in the large artery and small vein segments were small and variable.

Therefore it is concluded that serotonin acts directly on the coronary tree producing over-all vasodilatation. The site of maximum dilatation is in the segment between small arteries and veins.

93. AN INTENSIVE DAY-BY-DAY STUDY OF MINOR RESPIRATORY DISEASE IN TEN "HEALTHY" ADULTS

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A detailed and intensive clinical, epidemiologic, and virologic examination has been made of a small group of adults working together in proximity. For the 9-month period, September, 1958, through May, 1959, the participants in this study were interviewed daily concerning possible symptoms; throat swabbings were obtained thrice weekly from these individuals for possible virus isolation. Blood for antibody studies was obtained every 4 weeks.

Results of this investigation included the demonstration of a very low rate of absenteeism and yet a surprisingly high incidence of minor symptoms. The average number of symptom-free days per person was only 120 out of 266 days, or only 45 per cent of the time. Thus, more often than not, the average subject in this group was symptomatic. Most symptoms were referable to the respiratory tract. It was possible to identify and demarcate discrete episodes of afebrile respiratory disease. These episodes varied in number from 2 to 10 per person for a mean of 4.5 per person. Symptoms referable to the gastrointestinal tract were far less frequent yet they comprised the next largest category of discrete minor illnesses (2.7 per person).

Examination of more than 400 throat swabbings, most of them selected from instances of overt illness, has not resulted in the isolation of virus. Serologic studies have demonstrated significant increases in antibody to influenza B virus in 2 subjects prior to the recognition of this disease in the general population.

Thus, this intensive study of 10 "healthy" young adults had demonstrated: (1) the surprisingly high frequency of minor symptoms as revealed by careful day-to-day questioning; (2) the occurrence of approximately 7 discrete minor illnesses per person, the majority of them involving the upper respiratory tract; (3) the afebrile nature of these illnesses and the low rate of absenteeism associated with these illnesses; and (4) the failure to categorize by the best of current virologic methods any but 2 of these illnesses.

These results suggest that the burden of minor disease is perhaps even greater than suspected and that most of it continues to defy etiologic definition.

94. REGULATORY EFFECT OF THYROID HORMONE ON CORTISOL METABOLISM

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(INTRODUCED BY WESLEY W. SPINK, M.D.)

The rate of production of cortisol was determined in dogs before and after intravenous injections of thyroxine analogs, triiodothyroacetic acid ("Triac") and triiodothyronine (T_3). Cortisol assays were made on blood obtained directly from the adrenals by means of indwelling catheters in the adrenal veins. Cortisol output increased 30 to 120 minutes following "Triac" and T_3 to the same magnitude as was produced by intravenous corticotropin. Dogs were pretreated with T_3 and infused with cortisol, which as the Porter Silber chromogens, disappeared from the plasma at nearly twice the rate as in untreated controls.

Removal from the plasma of exogenous cortisol was similarly accelerated in 4 patients having thyrotoxicosis in comparison with euthyroid controls.

Urinary cortisol metabolites measured as 17-hydroxycorticoids (17-OHCS) in two patients pretreated with T_3 did not reflect quantitatively the rapid turnover rate of cortisol. Urinary excretion of 17-ketogenic steroids (17-KGS) paralleled the increased turnover rate trend in the controls. Saturation of the Δ^4 – 3 ketone groups and subsequent conjugation are the principal metabolic transformations of cortisol excreted in the urine as 17-OHCS. The 17-OHCS account only for a small portion of the 17-KGS in the hyperthyroid state, the major component being C_{20} reduced metabolites.

Administration of T_3 to a partially adrenalectomized patient precipitated acute adrenal insufficiency. 17-KGS excretion in urine was constant and 17-OHCS excretion was diminished.

These observations permit the following conclusions: (1) The turnover rate of cortisol was accelerated in the hyperthyroid state. (2) Induced hyperthyroidism in a patient with limited adrenal reserve precipitated adrenal insufficiency because the rate of cortisol inactivation exceeded cortisol synthesis. (3) The major portion of urinary cortisol metabolites are more the highly reduced steroid conjugates.

95. STUDIES ON THE METABOLISM OF KIDNEY TRANSPLANTS

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The surgical methods for transplantation of human kidneys are adequately developed. Cures of terminal uremia have been achieved in a number of cases of identical twins by transplantation of a kidney from the healthy partner to the other. This is possible because of the antigenic similarity between isogenic subjects. At present the major problem is to learn why grafts between *unrelated* individuals are destroyed. It is almost certainly established that an immunologic mechanism is responsible for these rejections.

Our studies with transplanted dog kidneys failed to show any correlation between the rejection process and the following measures of intracellular metabolism: (1) The ability of renal cortical slices to accumulate PAH against a concentration gradient from the medium was unimpaired in transplanted tissue removed from specifically immunized hosts. (2) The oxygen consumption of such slices does not differ from that of controls. (3) No significant alterations were observed in the rate of turnover of P^{32} in various fractions (acid soluble, lipid and nucleic acid) obtained from renal transplants. These metabolic measurements suggest that an essentially normal intracellular structure persists even at the time of rejection.

Since no metabolic changes within the cell were observed, we therefore suggest that the transplant rejection may not be an intracellular process. The reaction could involve the cell wall or the interstitial tissue. Preliminary experiments with rat grafts indicate that the antigenic substance is localized in a cell-free preparation which is rich in connective tissue.

96. OBSERVATIONS ON THE SITE OF INTESTINAL ABSORPTION OF COBALT^{60}-LABELED VITAMIN B_{12} IN THE MALE ALBINO RAT

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Our study was undertaken to determine the site of maximal absorption of radiocobalt-labeled vitamin B_{12} in the male albino rat. In the first part of this investigation, the animals were sacrificed at intervals of 15 minutes to 4 hours after ingestion of a measured oral dose of Co^{60}-labeled vitamin B_{12}. The small intestine was then removed, washed, and divided into segments. Counts of the radioactivity in each segment of the intestinal wall disclosed a definite peak of concentration in the upper and mid ileum.

To make certain whether this region of the bowel was the site of more efficient absorption of vitamin B_{12}, a second series of experiments was performed on surgically prepared animals. The percentage absorption of a given oral dose of Co^{60}-labeled vitamin B_{12} in normal rats was compared with the percentage absorption of the same oral dose in rats which had had resection of the upper half, the middle half, and the lower half of the small intestine, as well as in rats which had had right hemicolectomy and ileocolostomy. Absorption was estimated by measurement of both fecal excretion and urinary excretion after a flushing dose. The rats with the upper or lower halves of the small bowel resected, as well as the rats with ileocolic anastomosis, showed a percentage absorption within the range of the normal controls. The rats which had had resection of the middle half of the small intestine, however, showed very definite impairment of absorption as measured by fecal and by urinary excretion. The zone resected in these latter animals corresponded to the zone of maximal concentration in the bowel wall, as observed in the initial part of this investigation.

We conclude that in the rat the upper and mid ileum are the site of most efficient absorption of vitamin B_{12}, and that resection of those parts of the bowel produces definite impairment of this function.

97. THE MEASUREMENT OF CENTRAL CIRCULATING BLOOD VOLUME FROM PRECORDIAL DILUTION CURVES IN NORMAL SUBJECTS AND PATIENTS WITH MITRAL STENOSIS

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(INTRODUCED BY W. H. PRITCHARD, M.D.)

Double-peaked, precordial dilution curves can be externally recorded after the intravenous injection of I^{131} serum albumin and analyzed for cardiac output (CO) and mean transit time (MTT) through the heart and lungs. The purpose of the present study was to employ this technique to measure the central circulating blood volume (CCBV) by the Stewart-Hamilton method, $CCBV = CO \times MTT$, in normal subjects and patients with mitral stenosis at rest and after exercise.

In 25 resting normal subjects the mean CCBV was 1,080 ml., representing 610 ml./M^2 B.S.A. and 23 per cent of total blood volume. The CO and MTT averaged 5.84 L. per minute (index 3.30 L./M^2/min.) and 11.1 seconds, respectively. In 14 patients with mitral stenosis similarly studied, the CCBV was not significantly different, averaging 1,071 ml., 678 ml./M^2 B.S.A. and 27 per cent of total blood volume. The CO and MTT in these patients, however, was significantly different with means of 4.10 L./min. (index 2.57 L./M^2/min.)

and 15.8 seconds, respectively. Serial CCBV determinations were made in 8 subjects from each group before and after exercise (Master two-step test). In normal subjects there was no significant change in CCBV after exercise, while patients with mitral stenosis demonstrated a small, but statistically insignificant, decrease. Both groups showed a significant increase in CO and decrease in MTT after exercise.

The failure to demonstrate a difference in the CCBV between normal subjects and patients with mitral stenosis at rest is in agreement with other studies using similar dilution methods. Since this technique measures only that volume circulating during the primary curve and because of the probable failure of an unpredictable portion of pulmonary blood volume to participate in the primary dilution curve, particularly with pulmonary congestion, the Stewart-Hamilton technique must be questioned as an appropriate method of estimating this part of the CCBV.

The lack of significant change in the CCBV after exercise is discordant with the contention that the "reservoir" effect of the central venous volume is a primary determinant in the regulation of CO, and does not support the view that the relatively normal CCBV in mitral stenosis is secondary to a low CO.

98. PYRUVATE TOLERANCE TESTS IN HEALTHY SUBJECTS AND IN PATIENTS WITH DIABETES MELLITUS

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(INTRODUCED BY PAUL S. BARKER, M.D.)

Defective utilization of pyruvate in diabetes mellitus has not previously been clearly demonstrated. This can now be done by means of a precise intravenous pyruvate tolerance test. The test is based upon the observation that between 15 and 30 minutes after the intravenous injection of 10 Gm. (91 mEq.) of sodium pyruvate, the logarithm of the blood pyruvate increment (over the fasting level) decreases linearly against time. The regression coefficient ("b") of this line expresses numerically the rate of pyruvate disposal. All subjects were prepared with a standard high calorie high carbohydrate diet and were fasted overnight.

The mean regression coefficient from 24 healthy subjects was 0.0410 ± S.E. 0.0014. Mean values from 12 young (0.0407), 12 elderly (0.0413), 12 male (0.0413), 12 female (0.0407), and 6 obese (0.0407) subjects were identical. The mean regression coefficient from 16 untreated "adult" diabetic patients was 0.0312 ± S.E. 0.0013, and from five untreated "juvenile" diabetic patients was 0.0321 ± S.E. 0.0017. These values were significantly lower than normal ($p = 0.001$). Induction of normoglycemia with insulin injections did not significantly change the mean rate of pyruvate disposal in 11 "adult" (from 0.0322 to 0.0341) or in 5 "juvenile" (from 0.0327 to 0.0321) diabetic patients. In 9 "adult" diabetic patients treatment with metahexamide did significantly increase the mean rate of pyruvate disposal (from 0.0284 to 0.0344, $p = 0.01$). The low initial value for this group perhaps obscures interpretation.

It is concluded that there is a well-defined abnormality of pyruvate disposal in diabetes mellitus in man which is not related to coincidental factors such as age, sex, muscular development, diet, or obesity. This lesion is not corrected or improved after restoration of normal blood glucose levels by insulin injections. Modest improvement during sulfonylurea therapy is suggested. The data illustrate a fact which is evident from the clinical course of the disease: metabolism in diabetes is not fully repaired by exogenous insulin injections.

99. RENAL HEMODYNAMICS IN UNILATERAL AND BILATERAL RENAL ARTERY OCCLUSIVE DISEASE AND RESPONSE TO ARTERIAL REVASCULARIZATION

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For 20 years it has been recognized that hypertension resulting from unilateral renal disease could be benefited by nephrectomy. In recent years both cure of hypertension resulting from renal artery occlusive disease and preservation of the kidney has been made possible by reconstructive arterial surgery. Renal preservation is obviously paramount in patients with partial occlusion of both renal arteries. However, even in some instances of unilateral renal artery occlusive disease, renal salvage may be essential when the contralateral kidney has undergone irreversible arteriolar changes as a result of long-standing hypertension.

This study is based on observations of 10 patients with renal artery stenosis treated by definitive arterial reconstruction. Arteriosclerosis was etiologic in all cases and produced unilateral involvement in 3 instances and bilateral involvement in 7. Moderate to severe hypertension was present in every case. Marked depression in glomerular filtration as measured by inulin clearance was observed in only 2 patients and both demonstrated extreme bilateral narrowing of the renal arteries by aortography. Renal blood flow as derived from para-aminohippurate clearance was not markedly depressed in any of the patients with chronic renal artery occlusive disease.

The bypass graft principle was used in 8 cases to restore pulsatile blood flow beyond the occlusive process. In 2 cases with well-localized stenotic lesions in the proximal portion of the renal artery a patch graft was used to restore a normal lumen. Immediately following operation blood pressure was significantly reduced but usually did not reach normotensive levels for 2 to 3 months. Glomerular filtration returned to normal in the 2 patients with extreme depression before operation and showed no striking change in the other patients. Similarly renal blood flow which was not significantly depressed before operation showed no significant alteration after operation. Serotonin and catechol amine determinations were within normal limits before operation and remained unaltered after revascularization of the kidneys.

100. ENZYMATIC ACTIVITY WITHIN THE HUMAN HEPATIC LOBULE

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Diseases of the liver are characterized by a zonal distribution of pathologic lesions. To afford a basis for correlation of susceptibility to pathologic changes with metabolic activity, the distribution of 7 enzymes was studied in lobules of 4 normal human livers.

Liver biopsies from individuals without hepatic disease were immediately frozen in liquid nitrogen, sectioned, and then dehydrated at –40° C. After warming in vacuo to room temperature, representative areas of the lobule—portal triads, central and periportal zones—were dissected out at a magnification of ×72. Pooled samples weighing 0.2 to 1.0 μg were incubated in microtest tubes for quantitative measurements using the ultramicrobiochemical techniques of O. H. Lowry. The parameters measured were total protein,

desoxyribosenucleic acid (DNA), alkaline phosphatase (AP), phosphoglucoisomerase (PGI), glucose-6-phosphate dehydrogenase (G6PDH), lactic dehydrogenase (LDH), isocitric dehydrogenase (ICDH), malic dehydrogenase (MDH), and glutamic-pyruvic transaminase (GPT).

Comparison of central and periportal areas showed that activities of PGI, LDH, MDH and GPT were 10 to 25 per cent lower in the central zone. By contrast, the activities of G6PDH and AP were 16 to 28 per cent higher in the central zone while the activity of ICDH was equally distributed in the central and portal zones. The total protein and DNA were both 13 per cent lower in the central zone.

Analyses of portal triads dissected free of parenchymal cells contained only 12 to 45 per cent of the enzymatic activities found in the parenchymal zones. G6PDH was an exception and showed the triad activity to be almost 3 times the parenchymal activity. This higher activity was shared equally by arteries, veins, and bile ducts.

These data indicate that in the absence of histologic abnormalities, human livers exhibit zonal differences in enzyme activity. Previous studies on normal rat livers also demonstrated activity gradients between the lobular zones for these enzymes. However, when compared with the human data, species differences are demonstrated.

101. THE EFFECT OF DESOXYPYRIDOXINE INDUCED VITAMIN B_6 DEFICIENCY ON POLYUNSATURATED FATTY ACID METABOLISM IN HUMAN BEINGS

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Previous data from animal experimentation have shown that pyridoxine is intimately associated with the metabolism of polyunsaturated fatty acids (PFA). The vitamin is necessary in animals for interconversion of linoleic-linolenic and arachidonic acids and for the synthesis of fatty acids. The present study proposed to extend the animal observations into human beings, utilizing the techniques of experimentally induced vitamin B_6 deficiency. Seven subjects received 300 mg. of desoxypyridoxine orally each day while on a regular hospital diet. Six of the 7 subjects developed typical clinical pyridoxine deficiency. Blood samples withdrawn at intervals before, during, and after the deficiency state were divided into serum and washed red cell compartments and each analyzed for di-, tri-, tetra-, penta-, and hexaenoic fatty acids by the alkaline isomerization technique. In addition, serum total fatty acids, cholesterol, and phospholipids were measured when possible. The deficiency state was in all cases corrected by the addition of 150 mg. of pyridoxine to the experimental regime. To test interconversion, a load of 60 Gm. of corn oil by mouth was given at several intervals during the experiment and the immediate effects at 6 and 24 hours were measured. Three of the 7 patients showed a definite decrease in absolute values for the serum PFA which can be ascribed to the deficiency state; however, this decrease occurred in all components with no specific fatty acid patterns being found. Although the relative concentrations of the PFA in red cells differ greatly from serum, their pattern of fall was similar to that in serum. No consistent change occurred in the serum cholesterol or phospholipids due to vitamin B_6 deficiency. Corn oil produced an immediate marked rise in serum dienoic acids but little effect on other fatty acids. Pyridoxine deficiency did not significantly alter the response. None of the ingested fat entered the red cells since there was little change following the fat load. These original observations suggest that pyridoxine is essential for maintaining serum PFA levels presumably by

supporting fatty acid synthesis, but fail to confirm its role in interconversion. Red blood cells appear to be relatively inactive in fatty acid transport.

102. ANTIHYPERTENSIVE PROPERTY OF EXTRACT FROM RENAL MEDULLA

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Renal autoexplantation protects against renoprival hypertension when whole kidney or renal medulla is explanted to the peritoneum and when renal medulla is explanted to the lungs but not when renal cortex is explanted to the peritoneum (J. Clin. Invest. 38: 1027-1028, 1959). These results indicate that renal medulla contributes to the antihypertensive function of the kidney.

Based on the above observations an extract of renal medulla was prepared. Extracted medulla from two kidneys was made up to 100 ml. with Gey's solution and kept frozen.

Bilaterally nephrectomized dogs given a diet containing protein (3 Gm. per kilogram per day) and given sodium as physiologic saline or saline plus extract (2.5 mEq. sodium per kilogram per day) were used in all experiments. Two groups received the medullary extract daily intravenously. Two groups acted as controls. One control group had bilateral nephrectomy alone. The other group had nephrectomy and in addition received an extract of urinary bladder (autogenous) which was prepared and infused exactly as the medullary extract.

Nephrectomy alone (38 dogs) was followed by hypertension. There was no difference in the arterial pressure of the group receiving an extract of urinary bladder and the group having nephrectomy only (p 0.6).

One group of 11 dogs received once daily 2.5 ml. per kilogram of medullary extract. At 4 days the average change in arterial pressure was +11 mm. Hg. The fitted slope of the change in arterial pressure was different from that of the group having nephrectomy only ($0.05 > p > 0.025$). The slope was also different from zero (p 0.001).

Another group of 7 dogs received daily 5 ml. per kilogram of medullary extract. At 4 days there was no change in the average arterial pressure as compared to the control, although the slope fitted to these data was slightly negative. The fitted slope of the change in arterial pressure was different from that of other groups (p 0.001). Four dogs of this group were maintained without hypertension for 7 to 9 days.

An extract of renal medulla appears to protect against renoprival hypertension. The results seem to support further the view that renal medulla contributes to antihypertensive function(s) of the kidney.

103. DETECTION AND ASSESSMENT OF MITRAL REGURGITATION USING LEFT ATRIAL DILUTION CURVES

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In the presence of mitral regurgitation a portion of an indicator (the regurgitant fraction) is detectable in the left atrium immediately after its injection into the left ventricle. The reliability of this method has been studied by investigating the effect on the regurgitant fraction of varying the type and

position of the injection into the left ventricle and the position of the sampling catheter in the left atrium. The studies were done on anesthetized dogs with closed chests with or without chronic mitral regurgitation created either by tying chordae tendineae against the ventricular wall or by punching a circular hole 2 to 6 mm. in diameter in the anterior leaflet of the mitral valve. Using catheters introduced via peripheral vessels, dilution curves were recorded simultaneously from the left atrium and femoral artery following injections into the left or the right ventricle. Pressures in the left and right ventricles, left and right atria, and a femoral artery were also recorded. These pressure pulses as well as dilution curves recorded from the femoral artery were not significantly different from those in the control dogs. However, immediately appearing dye was detected in the left atrium in all dogs with mitral regurgitation and none or only minimal amounts in the normal animals. The average area of the defect in the anterior leaflet at necropsy was 8 square mm., and the associated average regurgitant fraction, 33 (10 to 60) per cent.

Regurgitant fractions after injections extending over several cardiac cycles or the systolic, diastolic, or full period of a single cycle were similar. Varying the position of the injecting catheter in the left ventricle had relatively little effect. However, position of the left atrial catheter must be verified by biplane roentgenograms since sampling from sites remote from the mitral valve or in pulmonary veins was occasionally associated with significant reductions in the measured regurgitant fraction. Positions near the valve and in the mid-left atrium yielded comparable results. The average difference between 92 replicate measurements of the regurgitant fraction was 2.6 (0 to 12) per cent.

It is concluded that this method is of practical value for the detection and assessment of mitral regurgitation.

104. ADENOSINE TRIPHOSPHATE IN HUMAN LIVER

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In an attempt to evaluate some of the biochemical alterations in the metabolism of pathologic human livers we have been studying the distribution of nucleotides prepared from surgical biopsy specimens. To date, over 30 specimens have been obtained. The nucleotides have been fractionated by column chromatography, identified and semiquantitated by a combination of paper chromatography and spectrophotometric measurements.

On the basis of these determinations we have found that specimens obtained from patients with fatty livers, hepatic cirrhosis, and chronic hepatitis are characterized by a marked diminution in the content of the nucleotide adenosine triphosphate. Since this biochemical lesion could arise from an increased activity of the mitochondrial enzyme adenosine triphosphatase in liver due to mitochondrial damage, we have determined ATPase levels of some of the same specimens which have shown decreased ATP content by chromatography. The results have shown that in these diseased livers the ATPase levels are significantly higher than in nonpathologic livers.

We believe our results are compatible with the hypothesis that in cases of fatty liver, hepatic cirrhosis, and chronic hepatitis the membranes of the liver mitochondria have been damaged, which leads primarily to a decrease in the adenosine triphosphate content of the liver as a result of increased ATPase activity.

105. BUTTER, CORN OIL, AND FIBRINOLYSIS IN RATS

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(Introduced by Samuel B. Grant, M.D.)

In previous experiments arterial thromboses and infarcts of hearts and kidneys were found in approximately one-fourth of rats fed an "atherogenic" diet modified by the addition of large amounts of butter. The essential ingredients of this "thrombogenic" diet are cholesterol 5 per cent, sodium cholate 2 per cent, thiouracil 0.3 per cent, and butter 40 per cent, the remainder being made up of casein, sucrose, vitamins, and salts.

Thrombelastographic studies of plasma clotting and clot lysis were performed on three groups of rats (20 in each group), one fed the thrombogenic diet, another fed a similar diet with corn oil substituted for the butter, and the third fed a "normal" semisynthetic diet. Rats on the thrombogenic diet had prolonged clot lysis times as compared to the other two groups. The clotting times of both groups on high fat diets were significantly shorter than those of animals fed the "normal" diets. These results suggest that thrombosis in rats fed the thrombogenic diet is the result, at least in part, of alterations of hematologic factors affecting thrombosis and thrombolysis.

106. "PSYCHOSOMATIC" DISEASE IN PHYSICIANS: AN ANTEROSPECTIVE STUDY

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The 660 freshman students who entered the University of Illinois College of Medicine in 1946, 1947, 1948, and 1949 were, upon their admission, interviewed by a psychiatrist and completed a Rorschach test. In 1957, a questionnaire requesting detailed information about personal and family health and type of postgraduate experience was mailed to all 632 who graduated. Each physician was asked to rate himself on a 4-point scale with respect to life and career satisfaction. Exactly 90 per cent responded. Results were tabulated anonymously. Data on the occurrence of four common "psychosomatic" disorders—duodenal ulcer, migraine, essential hypertension, and bronchial asthma—on the family history of these disorders, and on degree of life and professional satisfaction were abstracted. The psychiatrist who had examined these men a decade earlier was given a list of 75 names, 15 "normals," and 15 men each with ulcer, asthma, migraine, and hypertension. Kept ignorant of the medical data, he was asked to examine his interviews and Rorschach protocols, and to identify, by utilizing current psychiatric theory, the specific illness presented by each man and the "normals." The psychiatrist's predictions, family history data, and satisfaction rating scales were tabulated appropriately in 2 by 2 contingency tables and analyzed by the chi square test.

The psychiatrist was significantly more accurate than chance ($p < 0.05$) in separating the "normals" and the "psychosomatic" group. However, he was unable to distinguish those who developed one illness from those who developed another. There was a significantly higher incidence of ulcer, hypertension, and migraine, but not of asthma, in the families of physicians reporting the same illnesses, than among the families of physicians free of these disorders ($p < 0.05$). The group with peptic ulcer and hypertension but not

those with migraine or asthma reported a significantly higher incidence ($p < 0.05$) of major life and career dissatisfaction than those free of such illnesses.

The data suggest a complex interaction between heredity, pre-existing psychopathology, adverse life situations, and the occurrence of the so-called "psychosomatic" diseases.

107. THE EFFECT OF 3-(3-OXO-7 ALPHA-ACETYLTHIO-17 BETA-HYDRONY-4-ANDROSTEN-17 ALPHA-YL)-PROPRIONIC ACID LACTONE (SC-9420) ON EDEMATOUS CARDIAC PATIENTS

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The effects of SC-9420, an orally administered spirolactone, were observed in two markedly edematous patients with congestive heart failure. Both patients were resistant to mercurial diuretics and chlorothiazide.

SC-9420 alone temporarily increased the 24-hour urinary sodium from 1 to 45 mEq. in one patient. Urinary potassium excretion did not change significantly. Hydrochlorothiazide alone increased the urinary sodium excretion to 40 mEq. per day. When SC-9420 and hydrochlorothiazide were given together, urinary sodium excretion increased to 270 mEq. per day. SC-9420 potentiated chlorothiazide in a similar fashion but did not potentiate mercaptomerin. The second patient showed only a slight response to SC-9420 alone, but the effectiveness of hydrochlorothiazide was increased.

In both patients, the effects of SC-9420 itself were transient. Increasing the dose from 400 to 1,600 mg. per day neither increased nor prolonged the drug's effects. Since the compound has been shown by others to inhibit the action of aldosterone at the renal tubular level, the data indicate either that hyperaldosteronemia is not a major factor in the genesis of cardiac edema or that inhibition of aldosterone may be only temporary. These studies suggest that the most important effect of SC-9420 is the sensitization of the kidney to diuretic agents such as chlorothiazide. Thus, SC-9420 may be a useful adjunct in the therapy of intractable congestive heart failure.

108. THE EFFECT OF GRANULOCYTOTOXIC DRUGS UPON THE INFLUX OF S^{35} L-CYSTINE AND L-METHIONINE INTO HUMAN LEUKOCYTES

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This study is part of a systematic search for a possible biochemical defect in leukocytes of persons who have had agranulocytosis. The parameter of cell growth investigated was the influence of drugs potentially toxic to granulocytes, upon the influx of S^{35} l-cystine and l-methionine into human leukocytes. In vitro studies were made by incubation of sequestrenized whole blood with increasing micromolar concentrations of the drug investigated, following which S^{35} l-cystine or l-methionine was added. The leukocytes were then isolated and examined for radioactivity. Within the dosage range investigated, chlorpromazine had no effect upon the incorporation of l-cystine or l-methionine into leukocytes of normal persons and patients sensitive to this drug. In vivo studies were made by oral administration of S^{35} l-cystine to normal persons,

mentally ill patients, and persons known to be sensitive to chlorpromazine following a period of administration of this drug. Radioactive leukocytes were obtained in every case, which showed that chlorpromazine does not block the uptake of l-cystine in the patients studied.

An unexpected finding in one patient deserves special mention. A 49-year-old Negro man developed agranulocytosis following treatment with ristocetin. In vivo studies at the time of hematologic recovery showed an influx of S^{35} l-cystine into leukocytes in an amount 6 times as great as that seen in normal persons. Six months after recovery, this sixfold increase in incorporation of l-cystine into leukocytes was demonstrated repeatedly, in vitro. Preincubation of leukocytes with ristocetin reduced the uptake of l-cystine to "normal" values. It is possible that agranulocytosis in this patient may in some way be related to a block imposed on a postulated compensatory influx of l-cystine by ristocetin.

109. LUPUS GLOMERULONEPHRITIS: THE EFFECT OF TWO THERAPEUTIC REGIMENS ON RENAL FUNCTION AND RENAL HISTOLOGY

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Two consecutive groups of patients with severe lupus glomerulonephritis were studied by serial renal biopsy and renal function tests. They were selected for study on the basis of the renal histology. Twelve patients in the first group were treated with small doses of cortisone (average 50 mg. per day) or without corticosteroid hormones. Sixteen patients in the second group were treated for at least 6 months with large doses of prednisone (average 47 mg. per day).

The two groups were comparable at the outset in the following respects: (1) duration of renal involvement, (2) presence and degree of renal failure, (3) amount of corticosteroid therapy previously administered. (4) Moreover, 24 individual histologic features were analyzed semiquantitatively in the initial renal biopsies of all 28 patients; the lesions in the two groups were comparable in severity and activity. In the first (low steroid) group all patients died, and serial renal function studies showed progressive deterioration to death in uremia (average survival 11.4 months). In the second (high steroid) group 5 patients died, three in renal failure (average survival 13 months); *the other 11 are alive,* and little or no further deterioration of renal function was observed (average follow-up 24.4 months).

In the low steroid group, sixteen serial histologic studies were made in 8 patients. Histologic progression of lupus nephritis occurred in all but two. Thirty-two serial studies were made in 14 patients in the high steroid group. Histologic progression was seen in only one. The severity of the lesions was unchanged in 7, and histologic improvement occurred in 6. Fibrinoid, "wire loops," local necrosis, karyorrhexis, hematoxyphil bodies, and hyaline thrombi were considered signs of activity of lupus nephritis. Serial studies showed continued activity of the lesions in the low steroid group. By contrast, activity was decreased or completely disappeared in the high steroid group in 13 of 14 patients studied serially.

We conclude that using large doses of adrenal corticosteroids deliberately for six months improved the prognosis of lupus glomerulonephritis and halted progressive deterioration of renal function. Moreover, progression of lupus nephritis was stopped in all but one case and large doses of prednisone made the lesions histologically quiescent.

110. HEMOLYSATE TESTS APPLIED TO THE STUDY OF THE HAGEMAN TRAIT

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A concentrated extract of platelets, when added to normal platelet-poor plasma unexposed to glass, causes no measurable consumption of prothrombin, whereas an extract of erythrocytes (hemolysate) brings about good utilization of prothrombin. On this observation a test has been devised by which a deficiency of PTC (factor IX) or of thromboplastinogen (factor VIII, AHG) can be estimated quantitatively. A standardized hemolysate reagent is added to native platelet-poor plasma in a silicone-coated test tube and the prothrombin of the resulting serum determined. Low consumption is obtained when either PTC or thromboplastinogen is deficient. On adding aged normal serum with the hemolysate, complete correction is obtained when PTC is deficient, but none if thromboplastinogen is the lacking factor. Normal plasma adsorbed with $Ca_3(PO_4)_2$ when added with hemolysate will correct the prothrombin consumption of plasma lacking thromboplastinogen because adsorbed plasma contains a normal concentration of the latter. It will have no corrective action on plasma deficient in PTC.

It was found that the hemolysate test gave low prothrombin consumption on plasma from a subject with the Hageman trait. The addition of normal serum with hemolysate resulted in good utilization of prothrombin. Fair correction was also obtained with serum from a patient with hemophilia B (PTC deficiency) and with serum adsorbed with $Ca_3(PO_4)_2$, but little with serum from his own blood. The latter was also found less potent than normal serum when tested on normal plasma with the hemolysate test.

These observations suggest that a subject with the Hageman trait is deficient in an agent needed for the activation of PTC. This latter factor has previously been shown to be in an inactive state in plasma. PTC becomes activated during coagulation, thus accounting for the high concentration of the active factor in normal serum. A lack of PTC which occurs in hemophilia B causes a serious impairment of hemostasis, but a faulty activation as manifested in a test tube in blood from a subject with Hageman trait is apparently physiologically innocuous.

111. THE NATURAL OCCURRENCE OF HEMOLYTIC STREPTOCOCCI IN NORMAL SCHOOL CHILDREN (A FIVE-YEAR STUDY)

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Little is known of the natural occurrence of hemolytic streptococci and the epidemiology of streptococcal infections in civilian populations, in contrast to the extensive data available as a result of studies done in the Armed Services. This study was designed to investigate over a period of years the following questions: (1) the carrier rates for hemolytic streptococci in a specific age group (ages 8 to 9) of children; (2) the serologic groups and types of these streptococci; (3) the persistence of change in groups and types during the time of observation; (4) the effect of socioeconomic and environmental factors on groups, types, and carrier rates; and (5) the effect of the presence or absence of tonsils on carrier rates. The results showed the carrier rates to be highest in the school in the best socioeconomic area in the first 2 years of the study, but in the last 3 study years no significant differences were observed. Cumulative

percentages of children with positive throat cultures ranged from 40 to 88 in the 5 years. Group A streptococci were recovered four times as often as the other nongroup A strains which were composed of groups C, G, B, and a few nongroupable. Approximately 52 per cent of all group A strains were typable in the first 2 years; thereafter barely a third were typable. The typable strains isolated most frequently were 1, 3, 4, 6, 12, 28, and 33. Types changed in each school and in each study year. The degree of crowding in the home and school and the yearly meteorologic conditions did not seem to influence carrier rates directly nor did socioeconomic characteristics of the children's homes. The effect of the presence or absence of tonsils on carrier rates was variable but in general those children with tonsils had higher carrier rates.

112. ECOLOGIC MENTAL ILLNESS—PSYCHIATRY EXTERIORIZED

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Human ecology deals with man's mutual relationships (1) with his environment, including his diet, and (2) with other persons. Ecologic factors in mental illness have been neglected on the first score and generally misinterpreted on the second.

One may remain relatively symptom-free for many years when adapting to chemical additives and contaminants of air, food, water, and biological drugs; chemical drugs; foods; tobacco and dusts to which susceptibility exists. But as this adaptation to regular exposures tapers off, victims become tired, achy, worried, forgetful, dull-witted, disinterested, irritable, confused, and preoccupied with one-track thoughts. They may be negative, asocial, seclusive, suspicious, paranoid, hostile, disoriented, regressed, and amnesic. They may hallucinate, confabulate, or be subject to delusions. A minority—including relatively more children and both earlier and acute stages in adults—become stimulated, irritated, self-centered, emotionally uninhibited, hyperactive and uncoordinated (drunklike). Intense cravings for foods, drinks, or smokes, or maniacal excitement, convulsions, or altered consciousness may develop.

When daily environmental causes of chronic reactions are avoided simultaneously, chronic psychosis-like syndromes give way to neurosis-like symptoms. These taper off as neurological, musculoskeletal, dermatologic, genitourinary, gastrointestinal, respiratory, or other localized manifestations take over. These disappear as one becomes nonadapted to avoided materials to which susceptibility persists.

Experimentally controlled single re-exposures then induce acute convincing reactions that demonstrate cause and effect.

Similar acute episodes occurring accidentally in chronically reacting patients following massive doses or re-exposures after chance avoidance are usually misinterpreted. For instance, the initial phase of hyperactivity and irritability culminating in an emotional upset involving *any nearby person* is usually regarded as the "cause" of the more delayed "hangover" phase. Or equally erroneously, a confused victim often dubs some innocent bystander as the scapegoat. Such a priori deductions easily become one-track thoughts—especially if officialized by a physician.

This presentation—an extension of behaviorism—interprets mental illness in terms of susceptibility, adaptation, and conditioning to external stimuli. Ability to demonstrate external causes of acute and chronic phases opens an experimentally oriented approach to a subject previously dominated by psychogenic and other alleged inner forces neither measurable nor demonstrable.

113. MECHANISM OF GROWTH FAILURE IN RATS WITH LESIONS OF THE HYPOTHALAMUS

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(INTRODUCED BY CARL V. MOORE, M.D.)

The influence of the hypothalamus on growth rate and on pituitary growth hormone secretion was studied. Electrolytic lesions of various sizes and positions were made in the hypothalami of 96 young male rats. Comparison was made with a control group of 96 sham-operated, pair-fed animals. The rate of length increase (nose-tail), tibial length at death, and weight of endocrine glands were measured. Brains were examined in serial sections. In certain subgroups plasma PBI was determined and bioassays were made of pituitary content of growth hormone (tibial epiphysial width method in hypophysectomized rats) and TSH (I^{131} uptake method in hypophysectomized rats).

Because of associated endocrine deficiencies, certain subgroups were given replacement therapy with physiologic doses of one or more of the following: thyroxine, testosterone propionate, corticosterone, DOCA, and vasopressin. Lesions destroying the median eminence, primary hypophyseal portal plexus, or pituitary stalk all induced moderate to marked but never complete growth failure. Such lesions caused significant decrease in pituitary size and a reduction in other endocrine functions (low PBI, small gonads, small adrenals); replacement therapy, however, did not restore growth to normal. Lesions elsewhere had little effect on growth rate even when associated with specific endocrine abnormalities, fever, or obesity. Growth hormone content of pituitaries of rats with large median eminence lesions is significantly lower than normal as is the content of TSH. Although these experiments suggest that the hypothalamus is involved in regulation of growth and of growth hormone secretion, the possibility cannot be excluded that the observed effects are due to nonspecific disturbances in pituitary blood flow.

114. THE MECHANISM OF ACTION OF GROWTH HORMONE AND HYDROCORTISONE ON PROTEIN SYNTHESIS IN STRIATED MUSCLES

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Previous studies concerning regulatory mechanisms of protein metabolism have been limited to measurements of the over-all rate of conversion of amino acids to protein. These measurements do not permit discrimination between the cellular penetration of amino acids, the intracellular amino acid pool, and the enzymatic reactions involved in their conversion to protein.

In the present studies, a biologic system was devised that permitted discrete analysis of these parameters under controlled conditions. Intact diaphragm preparations, obtained from hypophysectomized rats treated with growth hormone, hydrocortisone, or saline injections, were incubated in media containing a C^{14}-labeled amino acid (leucine or valine) in concentrations approximating those of normal rat plasma. After one hour of incubation, radioactivity was assayed in the medium and in the perchlorate-soluble and insoluble (protein) fractions of diaphragm. From these measurements, rates of amino acid penetration and incorporation into protein were calculated. Cellular penetration, independent of other metabolic transformations, was studied with the nonmetabolizable amino acid alpha-aminoisobutyric acid (AIB-1-C^{14}). The size of the intracellular pool of this amino acid was determined from the steady-state concentration gradient in the living animal under similar experimental conditions.

Growth hormone stimulated the rate of amino acid penetration to approximately 50 per cent above control levels, as measured with either essential amino acids or AIB. Concurrently, the amount of radioactivity appearing in protein was increased by 20 to 100 per cent. The size of the intracellular amino acid pool was not substantially altered, indicating that penetration and the incorporation rates were stimulated proportionally. Despite the striking effects on penetration of essential amino acids, the accumulation of radioactivity in the amino acid pool was of the same order of magnitude as under control conditions. Hydrocortisone did not exert a significant effect on either the penetration process or the amino acid pool size but caused a 20 to 40 per cent decrease in the amount of radioactivity that appeared in protein.

These data indicate that growth hormone not only stimulates the penetration of amino acids but also shunts them to sites of protein synthesis. Heterogeneity of the intracellular amino acid pool is also suggested. Hydrocortisone appeared to block the conversion of intracellular amino acids to protein.

115. THE MODIFICATION OF A MACROCRYOGELGLOBULIN BY PENICILLIN, PENICILLAMINE, AND CYSTEAMINE

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In approximately 20 per cent of cases with macroglobulinemia Waldenstroem the macroglobulins are characterized by cryo or cryogel properties. The resulting excessive increase of blood viscosity often leads to critical intolerance of cold. A patient suffering from macrocryogelglobulinemia and exhibiting marked cold sensitivity, bleeding diathesis, and frequent infections provided the experimental material. The macroglobulin was characterized by: 19 S_{20}; gamma globulin position; axil ratio 11.5:1 (dehydrated); constituting 38 per cent of total serum proteins (9.2 Gm. per cent); gelformation between 13°-20° C., Sia test 3+. The effects of penicillin G potassium, D, L-penicillamine and cysteamine (beta-mercaptoethylamine) upon the macrocryogelglobulin were studied. Changes of relative viscosity of serum (20° C.), thermo-sedimentation rates (5°, 15°, 25°, 37° C.), gelformation, Sia (euglobulin) test, and immunophoretic properties served as indicators.

Penicillin G and D, L-penicillamine added to serum resulted in a remarkable decrease of viscosity, abolishment of gelformation, and a negative Sia test. Addition of cysteamine to serum resulted in a dosage dependent decrease of viscosity. Double diffusion technique (Ouchterlony plates—Hitzig) revealed the disappearance of the macroglobulin precipitin line to reappear fused with the gamma globulin line. Penicillin G 10 million units given intravenously lowered the relative serum viscosity from 243 to 54.8 (443 per cent) within one hour and increased the previously low sedimentation rates sharply. Rapid rebound of viscosity occurred. The addition of Benemid (500 mg. 3 times a day) to penicillin enhanced this effect markedly (decrease of relative viscosity by 1,253 per cent within 2 hours) and delayed rebound of viscosity. Oral administration of D, L-penicillamine (500 mg. 3 times a day for 10 days) resulted in sustained decrease of viscosity, increase in sedimentation rate, abolishment of gelformation and negative Sia test. The bleeding diathesis, cold intolerance, and susceptibility to infections were eliminated. It is concluded that the compounds employed were responsible for abolishment of cryogel properties, dissociation of macroglobulins, and marked clinical improvement.

The biochemical implications and the possibilities of experimental and clinical application of these observations will be discussed.

116. A METHOD FOR EVALUATION OF CHANGES IN STROKE OUTPUT AND VASCULAR RESISTANCE BY ANALYSIS OF THE ARTERIAL PULSE

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During ventricular diastole, the fall in arterial pressure is a "decay" curve, i.e., the rate of fall is a function of the momentary pressure level. A semilogarithmic plot of the pressure fall-off against time produces a straight line, the slope of which is determined by the mean arteriolar resistance (R). The area under the arterial pressure curve (ΣP) during the entire beat is a measure of the force which perfuses the arterioles. The ratio: $\Sigma P/R$ is therefore a means for evaluation of the beat-to-beat changes in run-off of blood from the arterial tree. In a steady state when the heart rate is regular, this value equals the stroke output. This thesis was tested in anesthetized, thoracotomized dogs. The stroke output, measured by rotameter in the ascending aorta, was modified as much as threefold by rapid infusions or blood withdrawal, and by administration of vasoactive drugs. Mean arteriolar resistance was changed by hypoxia and by drugs. Stroke output calculated from the femoral arterial pulse contour as slope $\cdot \Sigma P$ varied in direct proportion with the values obtained by direct measurement, and passed through the origin. Thus, tripling of the stroke output also tripled the product: slope $\cdot \Sigma P$. Constriction of the aorta increased ΣP markedly but a decrease in slope resulted in a calculated constancy of stroke output; a situation confirmed by rotameter. Slight variations in the logarithmic pressure decay pattern indicate the presence of low-frequency standing waves which reflect the stiffness of the arterial tree.

117. CORRELATION OF ELECTROCARDIOGRAPHIC AND ANATOMIC FINDINGS IN RIGHT VENTRICULAR ENLARGEMENT

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The authors reviewed 1,328 electrocardiograms recorded from autopsied patients and selected for special study 118 cases whose tracings satisfied one or more of Sokolow and Lyon's criteria for the electrocardiographic diagnosis of right ventricular enlargement. Of the 118 patients whose electrocardiograms were diagnostic of right ventricular enlargement by the above authors' criteria, only 47 were found at postmortem study to have anatomical right ventricular enlargement. Sixty-five per cent of the tracings presenting one or the other of the criteria of $R_{V1} + S_{V5} > 10.5$ mm. and S_{V5} or $S_{V6} \geqq 7$ mm. were erroneously diagnostic of right ventricular enlargement.

Sokolow and Lyon's criteria of an $R_{aVR} \geqq 5$ mm., a depressed ST segment and inverted T wave with R wave of 5 mm. or more in V_1, an $\frac{R/S \text{ in } V_5}{R/S \text{ in } V_1} < 0.04$ were found by the authors to be superfluous as diagnostic signs of right ventricular enlargement for the reason that when any of these features were present there was also one or the other of the following findings: right axis deviation of Â QRS to the right of +110° or an R/S in V_1 amplitude ratio > 1. In the accompanying table, the comparative values of Sokolow and Lyon's (Am. Heart J. 38: 273, 1949), Milnor's (Circulation 16: 348, 1957), and the authors' criteria in the diagnosis of right ventricular enlargement are presented.

	TOTAL CASES DIAGNOSED RIGHT VENTRICULAR ENLARGEMENT BY ELECTROCARDIOGRAM	ANATOMICAL RIGHT VENTRICULAR ENLARGEMENT (PER CENT)	FALSE POSITIVE DIAGNOSIS (PER CENT)
Sokolow and Lyon	118	100.0	60
Milnor	40	54.6	30
Authors	49	76.0	32

The authors believe that their criteria constitute a practical solution to the problem of diagnosing right ventricular enlargement in the electrocardiogram in that these criteria detected this condition in 37 of the 47 cases with anatomical right ventricular enlargement and at the same time presented far fewer false positive diagnoses of right ventricular enlargement than did those of Sokolow and Lyon.

118. THE MECHANISM OF SHUNT PRODUCTION IN ATRIAL SEPTAL DEFECT

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In 18 subjects operated on for atrial septal defect and having only an A.S.D. of known size, the correlation coefficient between the left to right shunt and the size of the defect was 0.286 ($p > 0.1$). This would be even smaller if subjects with reversed shunts were subjected to operation. In 23 subjects with A.S.D. but no other known lesions, who had complete cardiac catheterization in which satisfactory pressure records were obtained in all four cardiac chambers as well as in the pulmonary and systemic arteries, correlations were calculated between the over-all left to right shunt and each of the following parameters: pressure gradient from L.A. to R.A., $r = -0.068$, $p > 0.1$; L.V. minus R.V. end diastolic pressure $r = +0.368$, $p < 0.1$; systemic minus pulmonary mean arterial pressure $r = +0.415$, $p < 0.1$; systemic minus pulmonary vascular resistance, $r = +0.463$, $p < 0.05$; L.V. minus R.V. systolic pressure, $r = 0.570$, $p < 0.01$, the fact that L.A. to R.A. pressure gradient did not correlate well must mean only that it is too small to measure accurately by these methods.

The data are thought to be compatible with the generally accepted hypothesis that the shunt in a subject with an A.S.D. of sufficient size that it does not obstruct flow depends upon the relative distensibility of the two ventricles. The distensibility appears to be related to the systolic pressure generated by the ventricle, hence apparently to the thickness of the ventricular wall.

119. EFFECTS OF A CHEMICAL INHIBITOR OF 11-B HYDROXYLATION ON THE URINARY STEROID PATTERN IN PRIMARY HYPERALDOSTERONISM

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The administration of SU-4885 (Ciba), which is 2-methyl-1, 2-bis (3-pyridyl)-1-propanone, to normal persons has been reported to decrease plasma and urinary hydrocortisone, to increase urinary 17-ketosteroids and tetrahydro S, and to result in the appearance of Comp S and a steroid chromatographically similar to DOC in the plasma. These observations have led to the concept that SU-4885 blocks the synthesis of 11-oxygenated steroids due to the inhibition of 11-B hydroxylation in the adrenal cortex.

In an effort to learn more about the effects of SU-4885 on the synthesis of aldosterone, this chemical inhibitor was administered to two patients who had primary hyperaldosteronism caused by adrenal cortical adenomas. Both patients exhibited decrease in urinary aldosterone and an increase in urinary 17-ketosteroids, tetrahydro S, and pregnanetriol. The changes in urinary tetrahydro E were minor. While no attempt was made to determine the presence of DOC in plasma or urine, balance studies in one patient suggested that an effective mineralocorticoid was present during the administration of SU-4885 when urinary aldosterone was greatly reduced. These observations indicate that SU-4885 is capable of inhibiting the synthesis of aldosterone.

120. STUDIES WITH PURE DECAPEPTIDE ANGIOTENSIN. I. GENERAL AND PULMONARY HEMODYNAMICS

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It has been implied by some that angiotensin (angiotonin, hypertensin), known to produce an increase in the pulmonary artery pressure on intravenous administration, plays no part in human essential hypertension. The conclusion has been based partly and indirectly on the normalcy of pulmonary artery pressure of hypertensive patients when not in left ventricular failure. The present study is a beginning attempt to clarify this aspect of the problem.

A double lumen catheter, the distal tip securely wedged, was introduced into the pulmonary artery of 5 normal individuals. Following the simultaneous determination of control central venous and brachial artery pressures, and cardiac output (Fick), angiotensin was administered intravenously for 32 minutes at a constant rate of 4 units per minute. Pressures were recorded every minute except from the 10th to 15th and 25th to 30th minutes, when the cardiac output was determined.

The oxygen consumption and cardiac output increased slightly. The average brachial and pulmonary artery pressures increased significantly and consistently between 40 and 43 per cent. The average wedge pressure similarly increased 150 per cent. The pulmonary artery-wedge pressure gradient remained unchanged. As a result, average systemic vascular resistance increased by 33 per cent, average total pulmonary resistance by 36 per cent, but pulmonary vascular resistance remained unchanged.

The mechanism of the rise in pulmonary artery and wedge pressures and total pulmonary resistance is currently unexplained. However, the systemic and pulmonary vascular resistance data imply a profound peripheral arteriolar constriction with no concomitant effect upon the pulmonary arteriolar bed. In this guise, the findings are not inconsistent with the view that angiotensin plays an active role in human essential hypertension despite an increase in the pulmonary pressure during an acute period of observation, when insufficient time has elapsed for the development of possible hemodynamic readjustments.

121. STUDIES ON THE EVALUATION OF TULAREMIA VACCINES IN MAN

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The ideal method of evaluating a vaccine intended for protection of human beings is to challenge immunized and nonimmunized volunteers with a reproducible known infective dose of the disease-producing agent. *Pasteurella*

tularensis represents an ideal organism for such studies because of its high infectivity and its sensitivity to streptomycin and other antibiotics. These studies have been divided into 3 phases. In phase 1, volunteers were challenged intracutaneously with 1 minimal infective dose for man. Local lesions were obtained in all controls as well as in all previously immunized with Foshay heat-killed vaccine. Acute constitutional symptoms characteristic of tularemia were observed in 11 of 12 controls, 5 of 19 immunized, and 2 of 8 previously infected controls who were rechallenged 2 to 8 months after first infection. In phases 2 and 3 volunteers received a minimal infective dose via the respiratory route by means of a reproducible aerosol. In phase 2, 6 of 8 controls as compared to 8 of 14 Foshay immunized volunteers contracted clinical tularemia. In phase 3, immunization was conducted by the multiple puncture method with a viable but attenuated variant of *P. tularensis*. In this part of the study 8 of 10 controls but only 3 of 18 immunized showed clinical evidence of tularemia. It is concluded from these studies that Foshay heat-killed vaccine reduces the incidence of constitutional signs, but not local lesions following intracutaneous challenge; however, the incidence or nature of infection via the respiratory route is not altered. On the other hand, living attenuated vaccine does show a significantly greater protective effect against respiratory challenge.

122. A UNIFIED APPROACH TO UNEVEN DISTRIBUTION OF BLOOD AND GAS IN THE LUNGS

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(INTRODUCED BY HARRY F. DOWLING, M.D.)

Most pulmonary diseases result in some degree of uneven distribution of blood or gas in the lungs. The consequences range from slight reduction of efficiency of gas exchange in mild cases to life-threatening hypoxemia and hypercapnea in advanced cases. Serious difficulty can develop despite the fact that ventilation and cardiac output both exceed normal levels because the area of the interphase between blood and gas is less than normal. There are different methods for approaching these disturbances of distribution, but none so far has described the whole picture. Therefore, a unified approach to the total problem of maldistribution is proposed. It is based on the concept that there are four basic defects of distribution which may result in an infinite variety of combinations and permutations. These are: (1) virtual venous admixture due to the perfusion of unventilated alveoli; (2) alveolar dead space resulting from ventilation of unperfused alveoli; (3) nonuniform distribution of inspired gas; and (4) nonuniform distribution of blood flow.

A protocol has been developed for measuring all of these factors so as to permit piecing together the total picture of distribution. It consists of three steps: (1) A single deep breath of O_2 is taken and the alveolar N_2 and CO_2 are recorded continuously with volume during expiration. (2) Arterial blood and mixed expired gas are collected simultaneously while the patient breaths room air. (3) Blood and gas are again collected after the patient has breathed 100 per cent O_2 for 10 minutes. Venous admixture, both anatomical and physiological, can be calculated from the alveolar-arterial PO_2 differences. The dead space, both anatomical and physiological, can be calculated from the (alveolar-expired) and (arterial-expired) P_{CO_2} differences. Uneven distribution of inspired gas can be quantitated in terms of the rate of change of expired alveolar N_2 after a single deep breath of 100 per cent O_2. Uneven distribution of blood can be quantitated in terms of the alveolar-arterial P_{CO_2} difference. The findings in various pulmonary diseases will be discussed.

123. EFFECT OF SEROTONIN AND HISTAMINE ON CONNECTIVE TISSUE IN THE RAT

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(INTRODUCED BY A. CARLTON ERNSTENE, M.D.)

Serotonin and histamine appear to be involved in allergic and anaphylactoid reactions but their relationship to connective tissue has not been clearly defined. This study was undertaken to determine the effect of serotonin and histamine on connective tissue in the rat. Two Ivalon sponges were implanted into the subcutaneous area on each side of the back in 200 Sprague-Dawley rats during light ether anesthesia. Serotonin or histamine in doses of 1 to 30 mg. was administered subcutaneously daily to equal numbers and examined at weekly intervals for 1 to 4 weeks. These animals were compared with 16 controls.

Serotonin in 2 mg. doses caused more inflammation in the sponges but did not alter fibroplasia as compared to controls. Heavily granulated mast cells were noted at the periphery of the sponges. Larger doses of serotonin slowed the rate of fibroplasia and inflammation appeared less than that seen in controls at 3 and 4 weeks. Histamine did not cause an inflammatory reaction greater than that seen in controls. However, fibroplasia was accelerated and frequently the sponge was filled in 2 weeks as compared to 4-week controls. Mast cells were consistently depleted of granules. Larger doses of histamine did not alter the reaction. When combined injections of serotonin and histamine were administered, fibroplasia was not accelerated as with histamine alone. When the dose of both biogenic amines was increased, the rate of fibroplasia was less than that seen in controls.

It is concluded that serotonin in small doses for periods up to 4 weeks does not influence fibroplasia in the rat. Larger doses impeded rather than stimulated connective tissue growth. Histamine stimulated fibroplasia. When these biogenic amines were injected simultaneously serotonin blocked the stimulating effect of histamine on fibroplasia.

124. THE DISTRIBUTION OF BLOOD FLOW IN THE KIDNEY OF THE DOG

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Qualitative changes in the distribution of blood flow within the kidney have been observed under various experimental conditions. Further progress depends upon quantitative measurements of tissue blood flow. The method used in the present work yields flow data applicable to volumes of 5 c.mm. The results indicate a consistent pattern of flow rates in cortex, medulla, and papilla.

The thermometric method for measurement of tissue blood flow has been previously described. Flow was studied in the left kidneys of 12 sodium pentobarbital-anesthetized dogs. Urine was collected from the left ureter. Fifty observations were made at various parenchymal sites. Each site was marked by the electrolytic deposition of iron, allowing for its subsequent exact location on mounted tissue sections. The capsule-to-site distance, expressed as a percentage of cortical thickness, then permitted correlation of the data in all dogs. Conventional clearance techniques, including extraction ratio of PAH, were simultaneously carried out as an index of left kidney function.

Average blood flow as determined by PAH clearance was 3.5 ml. per minute per gram of tissue. Assuming a ratio of 2:1 for cortical to medullary mass, the thermometric method yielded an average rate of 3.1 ml. per minute per gram.

Of primary interest is the pattern of flow throughout the kidney. In the cortex the flow rises progressively from the capsule to reach a maximum rate near the cortical medullary junction. Deep to the junction, the flow falls sharply to reach a stable low value throughout the outer medulla. In the inner medulla, the flow again falls.

The average flows (expressed in milliliters of blood per gram of tissue per minute) at various depths beneath the capsule (expressed as percentage of the cortical thickness with 100 per cent representing the cortical medullary junction), are: 0.60 at 10 per cent (near the surface), 6.00 at 80 per cent, 2.40 at 160 per cent (middle of outer medulla), and 0.24 at 240 per cent (middle of inner medulla).

125. THE HYPOGLYCEMIC EFFECT OF L [illegible]UCINE IN ORGANIC HYPERINSULINISM

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The authors have had the opportunity recently to study two patients with proved islet cell tumors of the pancreas and associated hyperinsulinism. In both cases the histories were of interest in that the signs and symptoms were of short duration and neither patient had gained weight or had changed her dietary habits.

The first patient, aged 39 years, had fasting blood sugars between 23 and 50 mg. per cent; the intravenous glucose tolerance test showed an extremely high glucose disappearance rate ($K_G = 4.62$). However, the patient was not refractory to intravenous glucagon-free insulin. Because of the findings of Cochrane and associates (J. Clin. Invest. 35: 411, 1956) in familial hypoglycemia, the effect of L-leucine was studied. After oral administration of L-leucine the patient promptly became semicomatose with a blood sugar of 5 mg. per cent. Glycine had no demonstrable effect. The fasting blood sugar returned to normal on adrenal steroid therapy and, on this regimen, L-leucine produced only a mild fall in blood sugar. On admission, urinary 17-hydroxycorticosteroid levels were high but returned to normal with better dietary control. At surgery a solitary pancreatic adenoma was removed and postoperatively was without effect on blood sugar.

The second patient, aged 76 years, showed fasting hypoglycemia and a normal K_G value (1.73) following intravenous glucose. Again, in this patient on two occasions oral L-leucine exacerbated the hypoglycemia while glycine was without effect. At surgery a metastatic islet cell carcinoma was found and postoperatively the hypoglycemic action of L-leucine persisted. She has been treated with $zinc^{65}$ but not enough time has elapsed for therapeutic evaluation.

The exacerbating influence of L-leucine on the hypoglycemia of these two patients with presumptively high circulating insulin levels is unexplained at present but seems worthy of further investigation.

126. LACK OF IMMUNITY FOLLOWING TETANUS

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Approximately 35 cases of established recurrent tetanus have been reported. The recurrence of tetanus in the same patient suggests that natural active immunity is not produced or is inadequate to protect in this disease. Three patients with tetanus were studied to establish the presence or absence of antitoxic immunity following recovery. Each had an injury which had become infected between 1 and 4 weeks before admission. Stiffness of the muscles of the jaw, neck, abdomen, back, and extremities was present in each case. Two patients had generalized intermittent tonic spasms. All patients were hospitalized for from 3 to 5 weeks and given continuous sedation. The wounds were cultured but *Cl. tetani* was not found.

Two patients who had received tetanus antitoxin and penicillin at the time of onset of symptoms were studied 3 and 6 months, respectively, after discharge for evidence of immunity to tetanus toxin. Using the mouse protection test for determining tetanus antitoxin, serum samples prior to and one week following the administration of each of three immunizing doses of fluid tetanus toxoid spaced at 4-week intervals were examined for tetanus antitoxin. A demonstrable titer was absent in all samples until one week after the second injection of toxoid and then rose sharply to protective levels one week after the third injection.

The third patient was not treated with tetanus antitoxin because of horse serum sensitivity. When he recovered one month after onset, serum samples contained no antitoxin. Three immunizing doses of tetanus toxoid were then given at 4-week intervals. Antitoxin was detected 4 weeks after the second dose and increased rapidly one week after the third injection.

The primary immune response found in these patients indicates that no measurable degree of antitoxic immunity was provoked by the toxin liberated during the disease, for had it stimulated an immune response, which had declined in the first two patients, the first toxoid injection should have provoked an anamnestic response. In the third patient there was no evidence of antitoxin immediately after recovery, nor did the first toxoid injection stimulate its production.

127. CIRCULATING INSULIN ACTIVITY AFTER GLUCOSE: RELATION OF INSULOGENIC RESERVE TO CLINICAL SEVERITY OF DIABETES MELLITUS

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Last year Metz showed (J. LAB. & CLIN. MED. 52: 929, 1958) that rising blood glucose stimulates directly proportional increase of insulin secretion from normal pancreatic islets. By contrast the present report indicates that, although diabetics given oral glucose showed much higher rises in blood sugar than nondiabetics, corresponding increments of circulating insulin activity were always subnormal. Blood glucose (Somogyi) and "plasma insulin activity" (Vallance-Owen rat diaphragm) were determined during fasting, and 1 hour after ingestion of 100 Gm. glucose, in 12 normal subjects; 9 elderly diabetics whose therapeutic response to Orinase (tolbutamide) was excellent (Orinase-responders); 6 elderly diabetics who responded poorly to Orinase (Orinase-nonresponders); and 5 severe juvenile diabetics.

Increase of Blood Glucose Above Fasting (milligrams per cent; mean ± S.E.M.).—Normals 54 ± 6; Orinase-responders 145 ± 10; Orinase-nonresponders 119 ± 10; juveniles 163 ± 24.

Fasting Plasma Insulin Activity (micro-units effective insulin concentration per milliliter plasma; mean ± S.E.M.).—Normals and all elderly diabetics showed significant fasting insulin activity, but juvenile diabetics exhibited none: normals 59 ± 19 ($p < 0.001$); Orinase-responders 103 ± 38 ($p < 0.001$); Orinase-nonresponders 36 ± 18 ($p < 0.005$); juveniles 1 ± 2.

Increment of Plasma Insulin Activity After Glucose.—Only normals and the mildest diabetics showed significant increases above fasting values: normals 514 ± 110 ($p < 0.001$); Orinase-responders 350 ± 103 ($p < 0.025$); Orinase-nonresponders 41 ± 35 ($p < 0.5$); juveniles 2 ± 2.

"Index of Insulogenic Reserve".—The increment above fasting insulin activity after glucose, divided by the respective milligrams per cent rise above fasting blood glucose level, showed a sharply falling ratio across the spectrum of carbohydrate tolerance: normals 9.52 (100 per cent); Orinase-responders 2.41 (25 per cent); Orinase-nonresponders 0.34 (4 per cent); juveniles 0.01 (0.1 per cent).

The data indicate that total insulogenic reserve is sharply reduced in diabetes mellitus even when apparent impairment of carbohydrate tolerance is minimal, that it drops progressively as the clinical condition worsens, and approaches zero in the "total diabetic."

128. A STUDY OF ADRENAL STEROIDS IN BLOOD AND URINE IN ACUTE AND CHRONIC RHEUMATIC FEVER

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Adrenal function during the course of rheumatic fever has been evaluated in children (Kelley and associates), but not in adults with this disease. The present preliminary report is concerned with the plasma levels of 17-OHCS and the urinary excretion of 17-OHCS and 17-KS of adult patients with acute, subacute, and convalescent rheumatic fever. The values are compared with those obtained in a small group of patients with other diseases of connective tissue.

The subjects included 29 patients with acute and 8 with convalescent rheumatic fever, and 7 patients with other connective tissue diseases (lupus, scleroderma, etc.). The ages range from 17 to 44 years. The diagnosis of rheumatic fever was established according to the criteria of Jones, and the usual indices of activity were utilized. Blood corticoids and 24-hour urinary excretion of 17-OHCS were determined by the Porter-Silber color reaction after enzyme hydrolysis (urine), and chloroform extraction. The 24-hour urinary excretion of 17-KS was determined by the Holtorff-Koch method.

Blood corticoids were normal in all subjects. Urinary excretion of 17-OHCS and 17-KS was depressed in 23 (79 per cent) patients with acute rheumatic fever. In 3, 17-OHCS excretion alone was depressed, and, in 3, the urinary excretion of both was normal. During convalescence abnormal excretory patterns reverted to normal. Urinary excretion of 17-OHCS and 17-KS was normal in 6 of 8 (75 per cent) convalescent patients; in 1, 17-OHCS excretion was depressed, and in 1, 17-KS excretion was depressed. Urinary excretion of 17-OHCS and 17-KS was normal in all subjects with other connective tissue diseases.

The pattern of a normal plasma level with depressed urinary excretion suggests either impaired renal excretory capacity for these substances, or conjugation or protein-binding of these steroids in blood so that they are not available to the kidney for excretion. This aspect of the problem is now under study.

The results of this study contrast with those in children of Kelley and associates who found initially elevated blood corticoid levels in early acute rheumatic fever, and abnormally low levels in established active as well as in inactive rheumatic fever. Their values for urinary excretion of 17-OHCS were normal.

129. TRYPTIC DIGESTION INTERFERED WITH BY ASTHMATIC SPUTUM

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(INTRODUCED BY ERNEST O. THEILEN, M.D.)

The extremely tenacious sputum characteristic of bronchial asthma is one of the chief problems in treating the disease. Since it has been demonstrated that various allergic reactions are accompanied by an increase in proteolytic activity with a balancing increase in antiproteolytic factors, it occurred to the investigator that antiproteolytic substances might in some way interfere with formation of normal bronchial secretions.

Sputum from more than 30 patients with characteristic asthma and sputum from more than 20 patients with other diseases were tested for interference with tryptic digestion of film strip using the method described by Burdon. The sputum was prepared by adding an equal volume of borate buffer and passing the mixture through the screen of a Swinny syringe filter 10 times. Various dilutions of sputum were tried. Whether or not antiproteolytic substances are responsible for the characteristic abnormality of the sputum, there was regularly demonstrated interference with tryptic digestion of film strip gelatin when asthmatics' sputum was added to the system and not when the sputum was obtained from patients with other diseases. The addition of detergent, or hyaluronidase or of small amounts of potassium iodide did not affect the phenomenon. Keeping the sputum at room temperature for 24 hours or at 4° C. for three months did not reduce its capacity to interfere with tryptic digestion.

130. COMPARATIVE ASSAYS OF PURIFIED SHEEP FSH AND HUMAN PITUITARY GONADOTROPINS

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The biological and chemical characterization of human pituitary gonadotropins (HPG) and the establishment of an international reference standard have been the objectives of much research. Despite great differences in methods of isolation and bioassay, slopes of log dose-response curves for normal male and postmenopausal HPG have been reported as identical by statistical analysis of data obtained in several laboratories (Albert and associates, 1958). Identity of slopes is consistent with identity of biological activity even though relative potencies may vary. The different potencies observed can be attributed in part to differences in isolation procedures and in amounts of inactive protein material.

Recently available, highly purified, sheep pituitary gonadotropin (NIH-FSH-S1) has been evaluated in this laboratory as a potential reference standard for clinical use in assays of HPG. The sheep FSH was simultaneously assayed with two HPG preparations from normal male urine (AMW and FMW) and with two preparations from ovariectomized and postmenopausal women's urine (OWU and FWU, respectively). Assays were made in 542 immature female white mice, the end point being the increase in uterine weight. Dosage of HPG, depending on relative potencies, ranged from 0.3 to 4.0 mg., while of NIH-FSH from 0.05 to 0.2 mg. Dose-response slopes for AMW, FMW, OWU, FWU and NIH-FSH were respectively 52.6 ± 6.2, 50.7 ± 3.2, 60.0 ± 4.2, 83.7 ± 13.6, and 41.2 ± 2.7, with lambda values between 0.127 and 0.214. Of lowest potency, FWU had the highest standard error and steepest slope. By statistical analysis of the data, HPG slopes were found identical except for FMW versus FWU. However, dose-response slopes of three HPG's were significantly different from that of NIH-FSH. Slope of AMW did not differ significantly from that of NIH-FSH, but the number of AMW assays was small. These biological differences may be due partly to differences in species of origin or in source of material (pituitary versus urine), or to alterations occurring during isolation and purification of the various gonadotropin preparations.

131. EPIDEMIOLOGIC ANALYSIS OF PATTERNS OF BLOOD PRESSURE AND WEIGHT EVOLUTION, AND HYPERTENSIVE AND ATHEROSCLEROTIC DISEASE DEVELOPMENT—30-YEAR FOLLOW-UP DATA ON THE LABOR FORCE OF A CHICAGO UTILITY COMPANY

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In cooperation with a Chicago utility corporation, an epidemiologic analysis was accomplished of blood pressure, weight, and cardiovascular-renal (CVR) disease in 784 men aged 50 to 59 years on Jan. 1, 1954, and approximately 2,000 persons aged 40 to 59 years on Jan. 1, 1958—the entire labor force in these age-sex groups at these times.

The majority of both these groups had been in this company's employ for 20 years or longer. Weight and blood pressure data were available on many, beginning with their teens or young adulthood. Extensive sociological data were also available. A statistical analysis was accomplished on the serial blood pressure and weight data collected over 30- and 20-year periods, from young adulthood to middle age on several hundred men. An average of eleven blood pressures per person was available for this epidemiologic study. Determination of mean blood pressure for the group as a whole revealed a moderate increase with age. However, determination of the slope (fitted line) of blood pressure over the years for each individual confirmed the fact (noted on empiric examination) that about 30 per cent of subjects with low normal pressures as young adults maintained these without any significant rise throughout middle age. Such persons had markedly lesser risk of developing coronary heart disease and other CVR diseases in middle age than men exhibiting a rise in blood pressure over the decades. It was also found that even a single random determination of blood pressure in youth was highly significant on an actuarial basis in predicting risk of developing hypertension, hypertensive heart disease, coronary heart disease and other CVR diseases in middle age. A low order correlation was noted between patterns of weight and blood pressure evolution over the decades. Obesity significantly increased the risk of both hypertension and

hypertensive heart disease in middle age. The findings of this study demonstrated—contrary to some recent reports—that a rise in blood pressure is not an invariable concomitant of aging and cannot be viewed indiscriminately as a normal phenomenon.

132. THE DANGER OF ACTH IN ADDISON'S DISEASE

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(INTRODUCED BY ROBERT C. HARDIN, M.D.)

Eight of 17 patients with adrenocortical insufficiency developed ill effects after receiving the ACTH test. In 4 patients, 2 of whom died, we believe that these ill effects must be attributed to the use of ACTH. One patient had diarrhea on the first day of ACTH stimulation and vomited on the third day. Another, with idiopathic adrenal insufficiency, became weak and vomited on the second day of ACTH stimulation. A 65-year-old man with generalized blastomycosis appeared chronically but not seriously ill, with increasing weakness, anorexia, nausea, pigmentation, and hypotension. He was given ACTH for two days. The test was recommenced one day later because of incomplete urine collection. He developed Addisonian crisis and died 14 hours after the fourth dose of ACTH. Post-mortem examination showed systemic blastomycosis with destruction of the adrenal glands. A 39-year-old man was chronically ill with clinical evidence of bronchiectasis, amyloidosis, and Addison's disease. During a 2-day ACTH test he seemed to improve, but 18 hours after the test he abruptly developed Addisonian crisis. He died 6 days later. Post-mortem examination showed bronchiectasis, amyloidosis, and loss of the cortex of the adrenal glands.

Thorn and his associates have given several thousand infusions of ACTH to patients with a great variety of diseases, but observed untoward reactions in 6 patients only. All had received ACTH previously, 5 had Addison's disease. The reactions, urticaria and pruritus or delayed fever, appeared to be manifestations of sensitivity, and three subsequently received purified ACTH intravenously without reaction. None of our patients had received ACTH before. We submit several explanations of the ill effects of ACTH in these patients. Continued ACTH stimulation may exhaust the partially functioning adrenals. Contaminants of the corticotrophin, especially TSH, may induce Addisonian crisis. Corticotrophin itself may induce salt excretion through the mediation of a salt-excreting hormone. None of our patients received fluorohydrocortisone.

We support strongly two recommendations of Thorn to prevent morbidity during the ACTH test. Normal saline should be the vehicle. Fluorohydrocortisone should be given, during the ACTH test, to all patients suspected of adrenocortical insufficiency.

133. STUDIES ON THE PATHOGENESIS OF IDIOPATHIC EDEMA

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From among 45 patients with edema of obscure origin, 6 women who have responded identically to various testing procedures form the basis of this report. The patients showed an average gain in weight from morning to evening of 1.5 Kg., which was significantly greater than the average gain of 0.4 Kg. intra diem in normal subjects. Diuresis at night caused reversal of the normal diurnal pattern of water excretion and the frequent complaint of nocturia. These observations and the remarkable amelioration of edema during hospitalization

led to the finding that the upright posture was etiologically related to the pathologic accumulation of fluid. While renal function showed no abnormality by conventional criteria, and a standard water load (20 ml. per kilogram) was excreted normally in the recumbent posture by all patients, only 40.5 ± 4.6 per cent of the administered load was excreted within 4 hours when the patients were ambulatory throughout the test. This result was significantly inferior ($P < 0.001$) to the excretion of 81.8 ± 3.7 per cent of the water load by 22 normal subjects under the same conditions in the upright posture. The postural defect in water excretion was corrected by alcohol (30 ml., 95 per cent), by a cervical cuff inflated to 15 to 30 mm. Hg for the 4 hours of the test, and usually by various vasoconstricting drugs. In separate experiments, water diuresis was induced by drinking 300 ml. water hourly in the recumbent posture. After several hours of sustained diuresis in this posture, standing up reduced the free water clearance and osmolal clearance sharply, the former usually to negative values, while the endogenous creatinine clearance showed negligible changes. The data are compatible with the possibility that, when the patients are upright, undue pooling of body fluids in the dependent extremities might result in excessive release of ADH, with consequent renal retention of water and edema formation. Reversal of the postural defect by vasoconstrictors led to the therapeutic use of dextroamphetamine, with improvement or complete loss of edema for as long as the treatment was continued (up to 12 months in one patient) in the 5 patients so treated.

134. ZINC EXCRETION IN CHRONIC ALCOHOLISM AND LAENNEC'S CIRRHOSIS

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(INTRODUCED BY RAYMOND O. MUETHER, M.D.)

An increased urinary excretion of zinc in Laennec's cirrhosis has been described by Vallee, who suggested that a conditioned zinc deficiency may be important in its pathogenesis. This study compares the 24-hour urine zinc excretion without albuminuria in normal subjects, patients with Laennec's cirrhosis, and noncirrhotic patients with chronic alcoholism. Zinc was determined by a colorimetric method using dithizone. The mean 24-hour urinary zinc excretion in 10 normal males was 386 ± 128 mcg. with daily variations between 9 and 117 mcg. The average 24-hour excretion in 7 cirrhotic patients was 1335 ± 741 mcg. and in 5 noncirrhotic alcoholic patients, 1128 ± 112 mcg. The difference in zinc excretion between normal persons and cirrhotic patients and between normal persons and noncirrhotic alcoholics was statistically significant ($p < .001$). Only two cirrhotic patients had zinc excretion in the range encountered in normals.

Seven normal males ingested alcohol in amounts from 4.6 to 182 Gm. over a 6-hour period with resultant blood levels of 14 to 180 mg./100 ml. Zinc excretion during ingestion and the following 18 hours was compared for each with the mean of his control values. Increases in zinc excretion ranging from 20 to 184 per cent occurred in 6, the other had a 12 per cent decrease. Four cirrhotic patients and two noncirrhotic alcoholics received 3.97 Gm. of alcohol intravenously over a 6-hour period with resultant blood levels of 8 to 24 mg. per 100 ml. The zinc excretion was similarly determined with the preceding 24-hour excretion serving as control. In three cirrhotics zinc excretion increased from 33 to 368 per cent and one decreased 20 per cent. One noncirrhotic alcoholic increased his zinc excretion 11 per cent and one decreased 36 per cent. With one exception, subjects increasing their zinc excretion after alcohol also increased the urinary concentration of zinc, suggesting that water diuresis alone did not account for this change.

These results indicate that abnormal zinc excretion occurs in cirrhotics and noncirrhotic alcoholics. Although the changes in zinc excretion produced by alcohol were inconsistent, two of four cirrhotic patients and two of seven normal persons more than doubled their zinc excretion after this stimulus.

135. STUDIES OF RENIN AND JUXTAGLOMERULAR CELLS, COMPONENTS OF A REGULATING SYSTEM IN THE KIDNEY

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The granularity of rat juxtaglomerular cells (the juxtaglomerular index) is normally 30. The extractable renin in such a normal kidney will cause a 37 mm. rise in blood pressure in a bioassay rat. If a clip is made to narrow one renal artery so that hypertension results, the juxtaglomerular index of the opposite kidney falls to 1 and there is not enough extractable renin in that kidney to produce any hypertensive response at all. If the clipped kidney is excised and the hypertension becomes "cured," the juxtaglomerular index in the opposite kidney rises to 27 and the extractable renin in that kidney also increases greatly so that it produces a 28 mm. rise in blood pressure in a bioassay rat. The renin content and the juxtaglomerular granules both virtually disappear during the hypertension and reappear in normal abundance when the hypertension is "cured."

In another experiment, one group of normal rats were fed chow containing 7.4 per cent NaCl. The other group was fed ordinary chow only. Both groups drank tap water ad libitum. The kidneys of rats on ordinary chow had an average JG index of 29 and an average renin response of 45 mm. Hg. The kidneys of rats on chow with 7.4 per cent NaCl had an average JG index of 15 and an average renin response of 27 mm. Hg. Thus the rats on the high salt intake had a very significant drop in both renin and juxtaglomerular granularity ($p < 0.02$). In these rats there was a striking correlation between the renin content and the juxtaglomerular index in the individual rat kidneys. Such a correlation suggests (but does not prove) that renin might be the secretory product of the JG cells. The decrease in juxtaglomerular granularity with the higher salt intake probably represents slower secretion by the JG cells.

136. THE INFLUENCE OF DIETARY FATS ON THE FATTY ACIDS OF BLOOD, ATHEROMATOUS AORTA, LIVER, AND DEPOT FAT IN RABBITS

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(INTRODUCED BY IVAN D. FRANTZ, JR., M.D.)

The influence of dietary fats on the fatty acid composition of atheromatous aorta, blood, liver, and depot fat was investigated in five groups of rabbits placed on the following diets for periods of 4 to 6 months: Group I, Purina Rabbit Chow; Group II, similar to Group I plus 1 per cent cholesterol; Group III, synthetic diet containing 20 per cent safflower oil; Group IV, similar to III, plus 1 per cent cholesterol; and Group V, synthetic diet containing 20 per cent "Hydrol," plus 1 per cent cholesterol.

Groups II, IV, and V showed three- to sevenfold increases over Group I, in serum total lipids and fatty acids and developed extensive atherosclerosis in the aorta. Individual fatty acids (up to 24 different ones) were determined by gas-liquid chromatography. The percentage of major fatty acids in the diets, sera, and atherosclerotic aortae grouped according to saturation and chain-length

was tabulated. The values represent the per cent of a given acid or acids in the fatty acid mixture obtained from the tissues. There was great similarity in the tissue fatty acids between Groups I and II and between III and IV. The perinephric fat and liver fatty acids in each group reflected the type of dietary fat in a manner similar to that seen with blood and atherosclerotic aortae.

These findings indicate that the fatty acid composition of the atherosclerotic aortae and other tissues investigated is greatly influenced by the type of dietary fats.

137. PRODUCTION OF GLUCAGON ANTIBODIES AND THE DEVELOPMENT OF AN IMMUNOASSAY FOR GLUCAGON

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(INTRODUCED BY CARLETON B. CHAPMAN, M.D.)

Clarification of the physiologic role of endogenous glucagon may well require development of a specific and sensitive method for its assay in body fluids. Measurements of "glucagon-like" activity by liver slice techniques yield results of uncertain validity. If glucagon, like insulin, were antigenic, an immunoassay of high specificity and sensitivity would be possible.

Rabbits were given monthly subcutaneous injections of glucagon (Lilly) in Freund's adjuvant, and control rabbits received either adjuvant alone, adjuvant containing "glucagon-free" insulin, or nothing. Antibody levels were measured by determining globulin-bound radioactivity by the Berson-Yalow paper radioelectrophoretic technique following incubation of the various sera with 10 mμg. of glucagon-I^{131}. In sera from all 3 control groups globulin-bound radioactivity averaged 6.5 per cent (4.8 to 8.8 per cent), whereas in sera from glucagon-challenged rabbits it averaged 73.7 per cent (45.9-87.3 per cent). Almost identical values were obtained by means of the Skom-Talmadge technique. These nonprecipitating antibodies are in the gamma-globulin fraction and combine and dissociate with glucagon-I^{131} at a rapid rate. There is no cross-reaction with insulin.

Preincubation of antiserum with varying amounts of unlabeled glucagon reduces proportionately the ratio of globulin-bound to free radioactivity (B/F ratio). B/F ratio plotted against the amount of preincubated unlabeled glucagon yields a curve from which the glucagon concentration of unknown specimens can be determined. A sensitivity of 500 μμg has been achieved.

These data indicate that, despite its low molecular weight, beef-pork glucagon is antigenic in rabbits. Standard curves of high specificity and in ranges of exquisite sensitivity have been constructed, and the feasibility of an immunoassay for glucagon has been established.

138. PANCREATIC FUNCTION IN CIRRHOSIS OF THE LIVER

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(INTRODUCED BY G. L. CURRAN, M.D.)

The relationship of alcoholism and Laennec's cirrhosis of the liver is well documented, but the relationship of alcoholism and pancreatitis is less well established. Autopsy studies of patients with alcoholic cirrhosis of the liver

have demonstrated a high incidence of pancreatic necrosis, inflammation, and fibrosis. It has also been noted that there is a high incidence of hepatic pathology in patients with acute and chronic pancreatitis.

Sixteen patients with documented cirrhosis of the liver were studied by means of pancreatic function tests. The tests included in this survey were: serial serum and urinary amylase determinations, serum amylase response to secretin stimulation, and duodenal drainage studies including measurements of volume of pancreatic secretion and determination of the maximum bicarbonate concentration and total quantity of bicarbonate in the pancreatic secretion as well as determinations of amylase and trypsin in the duodenal juices. In addition to these "direct" pancreatic function tests, determination of serum carotene concentration was done in all patients and determination of stool fat and nitrogen as well as glucose tolerance studies were done in most patients. A few patients had radioactive iodinated serum albumin absorption studies and starch tolerance studies.

Ten of these 16 patients with hepatic cirrhosis had at least two of the "direct" tests abnormal. Ten patients had abnormal serum amylase concentrations. Of 14 patients in whom urinary amylase studies are available, 5 had abnormal values on at least one of three determinations.

Eight of 16 patients had an abnormal increase of serum amylase concentration after secretin administration. Eleven of the 16 patients had at least one abnormality of duodenal contents after secretin stimulation. Ten of the 14 patients of this group with data available on stool fat analysis had increased fat excretion. Four of the 16 patients had low serum carotene concentrations.

The results of this study indicate that 62 per cent of patients with cirrhosis have abnormal pancreatic function.

139. THE PATHOGENESIS AND PATHOLOGY OF ANAPHYLACTOID (HENOCH-SCHÖNLEIN'S) PURPURA

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(INTRODUCED BY LEWIS W. WANNAMAKER, M.D.)

This investigation attempts to clarify the relationship of Henoch-Schönlein's (anaphylactoid) purpura to acute glomerulonephritis and acute rheumatic fever through an analysis of the incidence of streptococcal infection in a group of 42 children with anaphylactoid purpura, as revealed by throat cultures and serum antistreptolysin-O (ASO) titers, and by microscopic evaluation of biopsies of skin, muscle, and kidney from these children.

Evidence of recent streptococcal infection (positive throat culture for group A streptococci, or high or rising ASO titers) was found in 14 (32 per cent) of 37 children. This frequency compares with that described for hospitalized, nonrheumatic children and contrasts with the incidence of elevated ASO titers in acute glomerulonephritis and acute rheumatic fever in children (80 per cent). Further, although the incidence of renal involvement in this series was high (60 per cent had abnormal Addis counts), only 5 (12 per cent) of the children with renal disease had evidence of recent streptococcal infection. Thus, there was no positive correlation between the presence of streptococcal infection and the development of renal symptomatology.

Kidney specimens were obtained from 11 children by percutaneous needle biopsy. The characteristic abnormality found in the acute phase of the renal disease was focal glomerulitis with areas of hypercellularity and fibrinoid accumulation in 10 to 60 per cent of the glomeruli. Serial and late phase

biopsies revealed that the glomerular lesions healed by focal scarring. The lesions observed differed strikingly from the lesions of acute glomerulonephritis and resembled the glomerulitis of disseminated lupus erythematosus.

Biopsies of skin and muscle from 14 children showed an intense acute vasculitis of capillaries, with perivascular cuffing and invasion of the vessel wall by polymorphonuclear leukocytes, round cells, and eosinophils. Collagen was often disrupted, and the lumina of capillaries were occasionally occluded by fibrinoid material and endothelial swelling.

We conclude that anaphylactoid purpura (1) has a different relationship to streptococcal infection than acute rheumatic fever or acute glomerulonephritis and does not seem to be a variant of either and (2) is a systemic vascular disease related to lupus erythematosus.

140. THE EFFECT OF HYPERTHYROIDISM ON SKELETAL MUSCLE METABOLISM

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Skeletal muscle weakness is a well-recognized clinical manifestation of hyperthyroidism. Past studies in experimental animals suggested that the biochemical basis for the muscular asthenia was a reduction in the muscle phosphocreatine. This paper reports biochemical and histologic studies on the skeletal muscle of pair-fed rabbits made hyperthyroid with thyroxine. Red and white skeletal muscle, as represented by the soleus and gastrocnemius, was studied to determine whether hyperthyroidism has a differential metabolic effect on the two types of muscle. There is a significant difference in the concentration of high energy phosphate compounds and glycogen in control red and white skeletal muscle. The glycogen, creatine, creatine phosphate, and adenosine polyphosphate content of normal white muscle is always higher than that of red muscle.

In the hyperthyroid animal, glycogen is the only constituent which is decreased in both types of muscle. Creatine is significantly reduced in white but not in red muscle. The decrease in the creatine content of white muscle in hyperthyroidism is due to a reduction in free creatine. There is no decrease in creatine phosphate or in the per cent of phosphorylated creatine. Adenosine polyphosphate content is not changed in either type of muscle. The maintenance of normal ATP concentration despite decreases in other muscle energy sources reflect its key position in muscle contraction.

Hyperthyroidism caused histologic abnormalities only in white muscle. There was focal degeneration of fibers, sarcolemmal proliferation and macrophage infiltration. These pathologic changes may reflect the more severe biochemical alterations in white muscle.

These results differ from previous reports which suggested that the primary effect of hyperthyroidism on muscle was a decrease in the phosphocreatine content. The data reported here indicate that hyperthyroidism first affects the free creatine content rather than the phosphorylation of creatine. They further suggest that the stress effect of hyperthyroidism is conditioned by the functional type of muscle. Red muscle may be less susceptible to excess thyroid effect because of a different biochemical pattern of energy generation. The differential metabolic effect of hyperthyroidism on skeletal muscle may have clinical implications since human thyrotoxic myopathy selectively affects the proximal girdle musculature.

141. A HUMAN STANDARD CLOT: OBSERVATIONS AND MEASUREMENT ON ITS INDUCED DISINTEGRATION

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A clot was developed from which continuous readings of its fibrinolytic disintegration and testing of factors influencing it under standard conditions were carried out. Citrated plasma was added to the 0.4 mark of the siliconized graduated stem of a Bauer-Schenk protein tube and then clotted with $CaCl_2$. The solution to be tested was introduced into the calyx of the tube which was stoppered after a glass bead was added and space for an air bubble was allowed. The tube was rocked at 37° C. with excursions of ± 10 degrees from the horizontal and 4½ cycles per minute. The volume of the dissolved clot was read at desired intervals. The advantages are as follows: (1) Fibrinolytic agents are tested on a preformed clot with standardized nonvariable surfaces (8 mm.²), and dilution of the test solution by serum from the lysed clot is negligible. (2) In contrast to the fibrin plate method determinations of the progress of clot lysis are possible at any time. (3) The method lends itself to serial determinations.

The following observations were made: (a) Human urokinase in buffered saline induces a lysis which, when the volume of the digested clot is plotted against time, produces a straight line for about two days. The curve for urokinase in plasma bends earlier. (b) Replacement of urokinase after 5 minutes' contact with the clot by buffered saline alone induces a self-perpetuating fibrinolysis of the clot which goes on for days; there is a close relationship between speed of this self-perpetuating lysis and activity of the urokinase solution used. Replacement of urokinase by plasma alone prevents further lysis. (c) Synthetic compounds have been found which enhance the effect on the clot of urokinase dissolved in buffered saline or in plasma. They also enhance the self-perpetuating lysis after removal of urokinase when they are contained in the substituting saline or plasma. These findings indicate that (1) the presence of active plasminogen activator alone is required to induce lysis of human plasma clots by activating the plasminogen adsorbed in it, (2) this lysis continues under certain conditions after no more activator is available, and (3) both processes can be enhanced considerably by synthetic compounds.

142. THE BACTERICIDAL ACTIVITY OF HUMAN SERA AGAINST ESCHERICHIA COLI

Burton A. Waisbren, M.D., and Imogene Brown, M.S. (By Invitation), Milwaukee, Wis.

Recent clinical and laboratory experiences in the augmentation of resistance to bacterial infection by the addition of gamma globulin to antibiotic therapy led to this study of the bactericidal activity of human sera against *Escherichia coli*. Experiments were done to develop a test for the ability of an individual's serum to kill *E. coli*. It is hoped that this test will prove helpful in the study of humoral resistance to bacterial infection.

Data will be presented regarding the following: (1) the bactericidal activity of human sera against varying numbers of *E. coli*; (2) strain differences in the resistance of *E. coli* to the bactericidal activity of human sera; (3) significant differences in the ability to kill *E. coli* of sera drawn from different individuals; (4) the effects of pH, buffers, storage at various temperatures, rotation and medium on the bactericidal activity of human sera; (5) the range of the bactericidal activity against *E. coli* of sera from 110 white males ranging

in ages from 1 day to 88 years; (6) heat lability and some relationships to complement of the factor in human serum that kills *E. coli*.

These data resulted in a standard test for the bactericidal activity of serum against *E. coli* in which the bactericidal titer of human serum against approximately 100 *E. coli* organisms of moderate sensitivity to sera is determined. Serum from healthy white adult males usually kills this number of *E. coli* in a 1:16 dilution. This value will serve as a base line for further studies in which the bactericidal activity of the sera from various groups of individuals will be compared for the purpose of perhaps finding deficiencies of clinical importance that may be amenable to correction.

143. PHENYLALANINE TOLERANCE TESTS IN PATIENTS WITH LEUKEMIA

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Quantitative changes of plasma amino acid levels have been previously reported in patients (Kelley, J. J., and Waisman, H. A.: Blood 12: 635, 1957). In order to evaluate further the abnormal metabolism that occurs in patients with leukemia, a number of amino acid studies have been performed which demonstrate that phenylalanine, glutamic acid, alanine, and proline occurred in high amounts in patients with chronic lymphatic and chronic myelogenous leukemia. These data to be reported provide information on the ability of patients with leukemia to tolerate high levels of phenylalanine administered orally. The data indicate that concentration of phenylalanine and tyrosine in the plasma after oral administration of DL-phenylalanine or L-phenylalanine are much higher in leukemic subjects than in normal patients. Indirect evidence is obtained that the phenylalanine hydroxylase system is impaired in the liver of these patients. The L-isomer of phenylalanine was absorbed at a rate faster than that of the D-isomer. Evidence was obtained that the leukemic subject had inability to metabolize L-phenylalanine compared to the normal individual.

144. THE MECHANISM OF HEMORRHAGE IN THROMBOCYTOSIS

ROBERT L. WALL, M.D., COLUMBUS, OHIO

Uncontrollable hemorrhage is seen in patients with myeloproliferative diseases complicated by platelet counts of 10^7 per cubic millimeter and above. This is seen most frequently in myelofibrosis, chronic myelogenous leukemia, and polycythemia, especially if splenectomy has been performed. In the absence of thrombocytopenia these patients rarely have spontaneous bleeding, but once bleeding is initiated fragile clot formation prevents hemostasis.

Whole blood coagulation times are normal with prompt clot retraction followed within the hour by extrusion of erythrocytes from the clot, grossly resembling fibrinolysis. However, fibrin clots are not dissolved by plasma from such patients, and the extrusion of erythrocytes is abolished by reduction in the number of platelets. Graphically, this reaction is seen on thromboelastography by a short reaction time (r), an increase in the rate of clot formation (k), and by a marked increase in clot firmness (ma); this is followed within an hour by rapid narrowing of the maximal amplitude as seen in fibrinolysis.

Thromboplastin generation times are abnormal when the patient's platelet level is above 10^7 c.mm., but becomes normal on simply reducing platelet concentrations. Fibrin formation in the presence of thrombocytosis shows gross

fragmentation which disappears on reduction of platelet concentration by centrifugation.

All of these observations may be reproduced with similar high concentrations of platelets obtained from normal donors, showing that this defect is purely quantitative and not related to any qualitative changes in the platelets of our patients. Electron microscopy of fibrin formed from whole blood in the presence of heavy platelet concentrations suggests that the fragile clot seen in thrombocytosis is actually a defect in fibrin polymerization which may be abolished by reduction in the concentration of platelets.

145. STROKE VOLUME IN MAN DURING EXERCISE

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(INTRODUCED BY HOWARD B. BURCHELL, M.D.)

The effect of exercise on stroke volume in man is controversial, due partly to differences in methods of measuring the cardiac output, in the severity of exercise, and in the degree of training and posture of the subject. The present experiments were designed to examine these factors.

Cardiac output was measured in 4 healthy untrained young men by the indicator-dilution method. Cardiac index, when the men were supine at rest, was 3.3 to 3.9; when standing at rest, 2.7 to 3.5 L./min./M.^{2} Stroke volume index when they were supine at rest was 49 to 59 ml./M.^{2}, and fell by 27, 43, 43, and 45 per cent (average 37 per cent) when the subjects stood up. Exercise was performed by walking on a treadmill with a 12-degree tilt and measurements were made during the third minute. With light exercise (less than twofold increase in oxygen consumption; cardiac index 4.0 to 4.4 L./min./M.^{2}), the stroke volume index reverted to almost the same value as that obtained when they were supine and resting. It then remained fairly constant up to moderate exercise (fivefold to sevenfold increase in oxygen consumption; cardiac index 6.8 to 9.8 L./min./M.^{2}). With severe exercise (eightfold to tenfold increase in oxygen consumption; cardiac index 8.6 to 12.2 L./min./M.^{2}), stroke volume index remained unchanged in two subjects and in two it increased by 19 and 24 per cent.

Thus stroke volume for subjects standing at rest is about a third less than when subjects are lying supine. This reverts to the value while supine with even mild exercise. There is then little additional change until exercise becomes severe, when stroke volume may increase by about 20 per cent. This increase, though small, at maximal heart rates of 170 to 180 per minute permits an additional cardiac output of about 2 L./min./M.^{2}. Studies of many earlier workers during upright exercise using carbon dioxide or inert gases gave similar results. Studies during supine exercise using cardiac catheterization show that man, like the dog, does not appreciably increase stroke volume. The discrepancy can be explained by differences in posture and severity of exercise.

A similar pattern of stroke volume changes with exercise may be found in trained athletes and in patients with postural hypotension, although the stroke volume with the patient standing at rest may differ considerably from normal.

146. EPICARDIAL POTENTIALS IN RIGHT VENTRICULAR HYPERTROPHY

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In an attempt to define further the origin and significance of the various right precordial QRS configurations seen in established instances of right ventricular preponderance, direct right and left ventricular epicardial potentials have been recorded on 50 patients with congenital heart disease coming to corrective open heart surgery and five normal adults undergoing resectional pulmonary surgery. Anatomical breakdown of the congenital group includes: valvular pulmonic stenosis, 10; tetralogy of Fallot, 8; ventricular septal defect, 21; auricular septal defect, 7; and A-V communis, 4.

Considering only the instances of valvular pulmonic stenosis and tetralogy of Fallot, which are the classic examples of anatomical right ventricular hypertrophy, distinct rR′ patterns were consistently identified as arising from the high medial outflow tract of the right ventricle on epicardial studies, with RS or rS complexes arising from the trabecular surface and apex of the right ventricle.

The auricular and ventricular septal defects exhibiting advanced states of pulmonary hypertension yielded similar rR′ complexes over the right ventricular outflow tract, whereas those with normal right ventricular pressures, albeit variable degrees of left-to-right intracardiac shunt tended to show rSR′ complexes, although individual instances of rR′ patterns were seen. Interestingly, right ventricular epicardial potentials on the 5 normal subjects revealed an rSR′ pattern in 1, rSr′ in 3, and an rS complex in the fifth.

Thus, it appears that the rR′ pattern seen in the right precordial leads of patients with right ventricular hypertrophy arises from a relatively focal area of the high medial outflow tract of the hypertrophied right ventricle. It is felt that this pattern merely represents an exaggeration of the normal right ventricular potential and does not imply an anatomical bundle branch block defect. It appears that the apparent right precordial qR and pure R wave complexes previously held as classic examples of right ventricular hypertrophy are, in fact, instances of the rR′ complexes where the initial r wave is either lost in the iso-electric line or is fused on the R′ wave.

The general clinical concept of right ventricular preponderance will be summarized.

147. ABNORMAL CALCIUM BINDING ASSOCIATED WITH HYPERGLOBULINEMIA, CLOTTING DEFECTS, AND OSTEOPOROSIS: A STUDY OF THIS RELATIONSHIP

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Bleeding and clotting abnormalities occur in disorders characterized by hyperglobulinemia (dysproteinemias). A patient with multiple myeloma and one with hyperglobulinemia of unknown etiology who had severe proved osteoporosis were found to have a delay in the rate of conversion of fibrinogen to fibrin by thrombin (thrombin time). Both bled abnormally and had water precipitable euglobins, cryoglobins, and prolonged clotting times. Clots were bulky, and failed to retract normally in spite of normal platelets. Thromboplastin generation tests were normal. The second stage of clotting was not involved in the

defect. The defect was present in whole and defibrinated plasma. The abnormal thrombin time was corrected by the "in vitro" addition of an excess of calcium ion. The amounts required were far greater than the amounts known to shorten the thrombin time of normal plasma. Normal plasma thrombin time was inhibited by the addition of abnormal whole plasma or gamma globulin fractions of both plasma and urine proteins obtained from curtain electrophoresis; calcium and magnesium ions corrected this inhibition. The concentration of calcium in plasma was greater than that in serum. This difference could be accounted for by calcium analysis of the euglobulin fraction. Binding of calcium by the abnormal gamma globulin was further demonstrated by a calcium-staining dye applied to paper electrophoresis strips. This was not observed in normal or in other hyperglobulinemic patients. Ultrafiltrable calcium content of each plasma, before and after precipitation of euglobulins, was less than normal, also indicating abnormal calcium binding. The intravenous infusion of ionic calcium, in amounts sufficient to normalize the serum ultrafiltrable calcium concentration, improved the thrombin time, corroborating the in vitro observations. Calcium recovery studies on serum demonstrated calcium-binding ability and a release of abnormally high values on prolonged incubation. Ashing of the serum yielded a correspondingly high calcium.

Bivalent ions are essential for final fibrin formation. Evidence is presented that calcium was bound in vivo in sufficient amounts by abnormal proteins to alter the thrombin time. The resulting alterations in calcium concentration were probably responsible for the clotting defects. These alterations, plus abnormalities in the matrix, probably produced the osteoporosis.

148. EVIDENCE SUGGESTING DIFFERENCES BETWEEN BACTERICIDAL AND HEMOLYTIC COMPLEMENT

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Complement is usually defined as that property of normal serum which causes hemolysis of erythrocytes "sensitized" with specific antibody. It is generally assumed that all activities ascribed to complement (hemolysis, bacteriolysis, cytolysis, viral inactivation, phagocytosis, participation in hypersensitivity phenomena) are due to identical serum properties. Studies presented here suggest that this point of view is, at least in part, incorrect. Data will be shown indicating that activity of the third component of complement (C′3) in bacteriolysis and hemolysis is separable.

With starch-block electrophoresis complement activity migrated with the beta-globulins. Activity for the first (C′1), second (C′2), and fourth (C′4) components moved as a single peak. However, activity for the third component (C′3) was spread through many fractions and the peaks for hemolytic complement and bactericidal complement activity were easily separable.

Using DEAE-Cellulose columns with high flow rates, hemolytic and bactericidal complement activity for the third component was (C′3) distinctly separate. Hemolytic complement (C′3) activity was eluted at ionic strength 0.12 to 0.14, bactericidal complement (C′3) activity was eluted at ionic strength 0.17 to 0.20. The separate fractions containing either bactericidal or hemolytic complement (C′3) activity fulfilled standard criteria for such activity. Thus, each restored appropriate activity to serum depleted of C′3 by incubation with zymosan (R3) and the activity of each fraction could be destroyed by incubation with properdin-zymosan complexes.

Separation of bactericidal and hemolytic complement activity (C′3) is amenable to many interpretations. It is possible that there are two distinct serum substances: one for bactericidal, the other for hemolytic activity. Thus

the various functions attributed to complement might be related to a variety of different serum properties. Some might be shared, being used in common in all functions; others might be more specific, being used only in certain select functions. Should such differences between "complements" be found to exist generally, current concepts concerning complement and other similar serum substances may require revision.

149. DEFECTIVE THIAMINE UTILIZATION IN PATIENTS HAVING CHRONIC NEUROLOGIC DISEASE

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In 5 patients having advanced neurologic disease whole blood thiamine levels were found to be abnormally low, in spite of a normal dietary intake of thiamine. For 2 of the patients supplementary oral thiamine restored the blood levels to normal. For the other 3 oral thiamine given in doses up to 1 Gm. daily failed to raise the blood levels to normal. In these patients the blood levels were restored to normal by the parenteral administration of thiamine. They were given parenteral therapy for varying periods of time but failed to show clinical improvement. This could be explained on the basis that the anatomic lesions were irreversible.

The patients showed a variety of clinical symptoms and signs and did not present a uniform clinical syndrome. However, many of the findings in these cases, particularly neuropathy, muscular weakness, sensory loss, reflex changes, muscle atrophy, and even convulsions, are described in experimental thiamine deficiency. In our cases deficiency of thiamine could be considered as partial not total, inasmuch as there was some circulating blood thiamine. The possibility exists that the neurologic deficits may have been produced by partial thiamine deficiency existing over long periods of time. The low blood levels of thiamine could be due to increased destruction in the gastrointestinal tract, a defect in absorption, increased destruction or improper utilization after absorption, or increased excretion in the urine.

150. THE THROMBOPLASTIC ACTIVITY OF SOYBEAN PHOSPHOLIPIDS

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(INTRODUCED BY WILLIAM J. HARRINGTON, M.D.)

Many efforts have been directed toward preparation of a platelet substitute suitable for clinical use. Certain platelet phospholipids are effective in vitro and may prove to be of value in vivo. Difficulties in obtaining platelets for starting material have prompted investigations on other sources. Crude soybean phospholipids appear to have some effect in controlling thrombocytopenic bleeding in clinical trials done by others, and we have obtained similar results in preliminary clinical trials with soybean cephalin.

Because of the possibility that purified phospholipids might have greater clinical utility than crude preparations, attempts have been made to isolate the active phospholipids from commercial soybean phospholipid (Gliddex P). The lipid fractions obtained were screened in the thromboplastin generation test.

The thromboplastic activity resides in the alcohol-insoluble fraction. The commercial preparation was further fractionated by differential solubility in

organic solvents combined with column chromatography. Three major fractions were isolated: lecithin, phosphatidyl ethanolamine, and an inositol-containing phospholipid fraction. The first two fractions appear to be free of significant contamination with other phospholipids, while some of the inositol lipid fractions were contaminated with phosphatidyl serine and some with phosphatidyl ethanolamine.

When tested, in vitro emulsions of lecithin or phosphatidyl ethanolamine were inactive in replacing platelets. The inositol phospholipid fraction was active in concentrations of about 200 to 1,000 μg per milliliter. Mixtures of lecithin and phosphatidyl ethanolamine or lecithin and the inositol lipid fraction were inactive. However, emulsions of mixtures of phosphatidyl ethanolamine and the inositol lipid fraction were active in concentrations ranging from about 25 to 1,000 μg per milliliter. The greatest activity was present in the inositol lipid fraction eluted first during column chromatography, and this fraction was contaminated with phosphatidyl serine.

Other workers using different starting material have reported marked thromboplastic activity for phospholipids different from those studied here, and there is disagreement in the literature regarding the nature of the active compounds. Thus it would appear that thromboplastic activity may not be a property of any single phospholipid or phospholipid mixture.

151. THE COINCIDENCE OF LEUKOCYTE AND THROMBOCYTE ANTIBODIES

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(INTRODUCED BY CHARLES A. DOAN, M.D.)

A macroscopic test for the detection of leukocyte agglutinins in the sera of animals and man has been reported from this laboratory. A similar technique has been employed macroscopically and microscopically to detect platelet antibodies.

The development of high titer antisera for human platelets has been induced in rabbits. These sera cause agglutination, fragmentation, and lysis of human platelets, and have provided criteria for the detection of antibodies in human sera. The test has proved useful in studying idiopathic thrombocytopenia and in detecting isoantibodies developing as result of blood transfusions.

Sera of 26 patients with demonstrable leukoagglutinins have been tested for platelet antibodies. Twenty of these contain platelet agglutinins while none contain acquired erythrocyte antibodies. Sera of 3 transfused patients contain strong platelet agglutinins but no leukocyte antibodies.

Ten cubic centimeters of serum containing strong leukocyte and platelet agglutinins from a patient who had shown repeated severe transfusion reactions was administered to a human volunteer. Within 15 minutes of slow intravenous administration of this serum, the subject developed headache, chills, and a temperature of 102° F. Serial peripheral blood studies demonstrated significant leukopenia and thrombocytopenia.

152. A "SUCCESSFUL" STAPHYLOCOCCAL CONTROL PROGRAM WHICH FAILED

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Nosocomial staphylococcal infections, particularly in neonatal and post-partum patients, continue to constitute a major problem. The recent epidemics of staphylococcal infection in these patients have been caused almost exclusively by strains of staphylococci showing the 52/42B/44A/80/81 ("80/81") pattern of bacteriophage lysis. Control of epidemics has been possible by transferring the personnel who are carriers of "80/81" staphylococci away from nurseries.

In the spring of 1958, neonatal and post-partum staphylococcal infections reached serious proportions in our hospital. Approximately 70 per cent of these infections were due to "80/81" staphylococci. A control program including monthly surveillance of newborn infants and contact personnel by culture of the nares for "80/81" staphylococci was instituted. Personnel harboring "80/81" staphylococci were transferred. The rate of nasal colonization of infants with "80/81" staphylococci fell from 10-30 per cent to 2 per cent by the spring of 1959 (17 of 937 infants). Simultaneously the frequency of maternal breast abscesses decreased from 2 per cent to virtually none.

In June, 1959, the number of deliveries increased with resultant overcrowding and understaffing in the newborn nursery. A recrudescence of neonatal and post-partum infections occurred, 40 per cent of newborn infants developing pyoderma due to coagulase positive staphylococci. However, at this time there were no carriers of "80/81" staphylococci in the nursery and the rate of colonization of infants with the "80/81" strain at discharge was less than 2 per cent. It was found that 65 per cent of the staphylococci isolated from neonatal pyoderma or maternal breast abscesses were lysed solely by the narrow range 44A bacteriophage, while virtually none of the staphylococci were of the "80/81" type.

Thus, even in the face of overcrowding and understaffing in the newborn nursery, a staphylococcal control program which was directed primarily at control of carriers of one type, the "80/81," succeeded in eliminating this strain. However, the "80/81" type was supplanted by another antibiotic resistant strain of staphylococcus (44A) and the infection rate in neonatal and post-partum patients reached "precontrol" levels.

These observations may represent a preview of the future problems which may be anticipated in control programs directed against specific strains of staphylococci.

153. THE EFFECT OF PRIOR SENSITIZATION WITH FREUND ADJUVANT UPON EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS.

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(INTRODUCED BY MARK H. LEPPER, M.D.)

The production of experimental allergic encephalomyelitis (EAE) is facilitated by incorporating brain tissue antigen in Freund adjuvant. The necessity of adjuvant is indirect evidence that delayed hypersensitivity is involved in the pathogenesis of this experimental model. Tuberculin-like skin tests to various brain fractions have been elicited in EAE and several studies have suggested correlation between delayed skin tests and the encephalitis in both time

and severity. Skin tests have been most marked with heterologous brain tissue although positive results with homologous tissue have been irregularly noted. If both the delayed skin test and the encephalitis are dependent upon the same antigen, then skin tests should be elicited with homologous tissue in animals previously sensitized with heterologous encephalitogenic antigen.

To gain further insight into the mechanisms of Freund adjuvant in EAE, 28 white male guinea pigs were injected intracutaneously with whole fresh homologous cord suspension incorporated in adjuvant and 27 equivalent animals were treated similarly with rabbit cord. Fourteen of the former and 13 of the latter had received Freund adjuvant without antigen one month prior to the injection of the cord antigens in adjuvant. Of these 27, only 2 were paralyzed by the fifteenth day following cord immunization and the remainder were apparently normal. Eighteen remained well by the thirtieth day. Of the 28 guinea pigs which had not received prior sensitization to adjuvant, only 7 were normal by the fifteenth day and 4 by the thirtieth day. Rabbit and guinea pig cord were equally encephalitogenic.

All guinea pigs were skin tested with old tuberculin and 10 per cent emulsions of rabbit and guinea pig cords. As expected, reactions to old tuberculin were more marked in the presensitized group. Delayed reactions to rabbit cord were uniformly elicited in those animals injected with the same antigen and were of greater intensity in the presensitized group, which had the lower incidence of paralysis.

If there is direct correlative meaning between skin hypersensitivity and encephalitis, then delayed skin tests should have been elicited to the guinea pig cord; however, reactions to homologous antigen were negligible.

154. LIPIDS AND LIPID MOBILIZER HORMONE IN PREGNANCY AND THE NEWBORN INFANT

Chris J. D. Zarafonetis, M.D., Joseph Seifter, M.D. (By Invitation), David H. Baeder, Ph.D. (By Invitation), and John P. Kalas, M.D. (By Invitation), Philadelphia, Pa.

A lipid mobilizing hormone (LM) was discovered in the plasma of man and other species by Seifter and Baeder in 1954. These workers later isolated LM from the posterior pituitary of hogs, and further demonstrated that the LM concentration of plasma is greatly increased by the administration of cortisone or by the subjection of animals to various stresses. Studies in patients undergoing the stress of surgery have led to the delineation of an LM-mediated physiologic mechanism in man for the acute mobilization of lipids to the liver. The purpose of this investigation was to determine a possible further role of LM in connection with the known increase of plasma cholesterol during pregnancy. Preliminary observations indicated a definite relationship (Abstr. Clin. Res. 6: 265, 1958), leading to more extensive studies to be described.

Plasma cholesterol and fatty acid determinations were made on blood from the mother during pregnancy, at term, and post partum; on cord blood; and newborn infant blood. LM hormone was assessed by bioassay or in vitro inhibition of clearing factor activity.

Elevation of plasma cholesterol and fatty acids during pregnancy was reconfirmed. Increased plasma LM activity appeared in the second trimester or earlier, persisted through the remainder of gestation, and for as long as a month or more post partum. The observed temporal relationship of these

phenomena suggests that the elevation of maternal blood lipids during pregnancy is mediated via the LM hormone pathway. Cord and infant bloods held one-third or less the lipid values found in maternal blood at parturition. The LM content of cord and infant blood, however, was increased to the degree found in the respective maternal blood at the time of delivery. Thus, LM apparently crosses the placental barrier. The relatively low fetal lipid levels, therefore, indicate that the omental lipid depots of the infants were either depleted prior to birth, or there had been no opportunity for lipid accumulation during gestation. Necropsy experience reveals the omentum to be thin and translucent at birth, which is consistent with either interpretation.

One Manufacturer's Opinion

A Welcome Borrowing

Observing the success of quality control in industry, professional groups are asking why quality control techniques should not be applied to other areas. Recent events point up demands by leading scientists for this application—especially to those areas dealing with the safeguarding of human life.

Aside from the particular product or service offered by a manufacturer, long-term success is, after all, dependent on the guarantee of reliability and quality offered the consumer. Knowing this, industry has developed rigid quality control over production. Extensive testing, taking weeks or months, is undertaken before release of a product run. This testing serves a dual purpose. First, efficiency in manufacturing is maintained and guarded; secondly, the product or service offered is kept at a standard to satisfy the discriminating public.

In the clinical laboratory, advances made in specific instrumentation, methods, technique, and diagnostic reagents have, until recent years, greatly exceeded the overall quality control practiced over biochemical determinations.

It is not surprising that modern advances and antiquated control are functioning side by side in laboratory testing. *Adequate control measures have not been available.*

Now, two major steps have been taken to fill this void. First, the application of statistical measures of precision and of accuracy, and the routine use of the specific deviation yardstick are becoming widespread *in revealing* laboratory efficiency problems. Secondly, the common usage of two important standards-in-serum for biochemical testing, Versatol (normal) and Versatol-A (abnormal), with known absolute values in the normal and abnormal range, meet the demands of advanced laboratory analyses.

Combined with technical skill, these safeguards over laboratory testing add welcome new chapters to accuracy in clinical chemistry.

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Quality-control in clinical chemistry featured at scientific meeting

Clinical Pathologists, Chemists Eye Application of Industrial Advances to Laboratory

New York, N. Y., Nov. 12, 1958. Interest in clinical laboratory quality control was heightened today when, the second time this month, this subject was covered at the Symposium on Laboratory Instrumentation at the Hotel New Yorker. Stephen J. Koziol of Warner-Chilcott, in a paper on "Recent Developments in Increasing the Efficiency of Clinical Analyses", stated the approach used by his firm:

"The third ... part deals with the objective standards and the objective controls which may be applied by the conscientious laboratory worker—to assure that neither human fatigue nor instrument fatigue are giving rise to clinically invalid results. Reference standards—closely duplicating both normal and pathological clinical chemistry specimens ... have been developed to aid quality control within the clinical laboratory itself."

Two reference standards, Versatol and Versatol-A, which closely duplicate both normal and pathological clinical chemistry specimens have been developed to aid quality control within the laboratory itself.

Earlier, in this same Symposium, pioneers in microchemistry recommended Versatol and Versatol-A for the *standardization* of the newest microchemical methods.

Quality Control in Clinical Chemistry was a featured presentation earlier this month at the American Association of Clinical Pathologists meeting in Chicago. The use of freeze-dried reagents *like* Versatol and Versatol-A was recommended for routine daily control.

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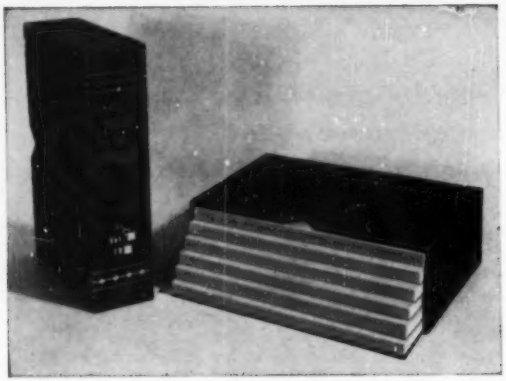

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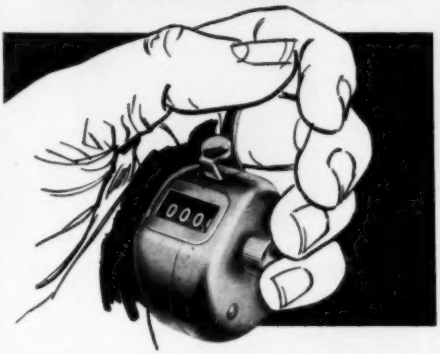

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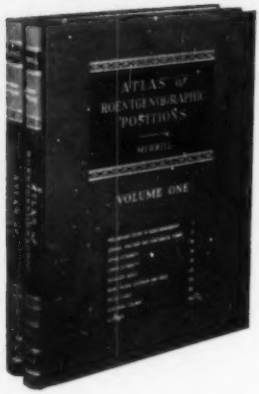

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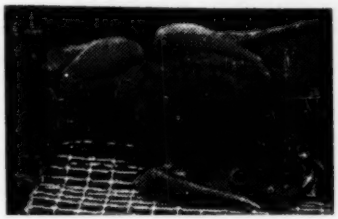

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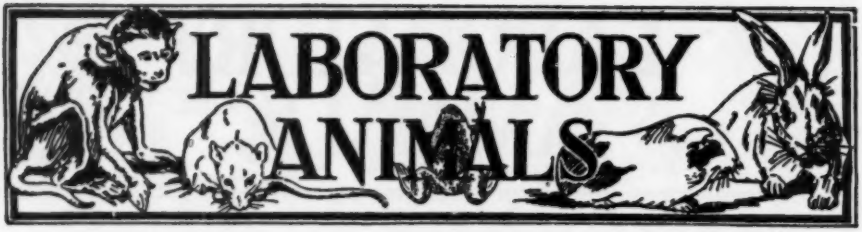

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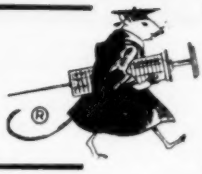

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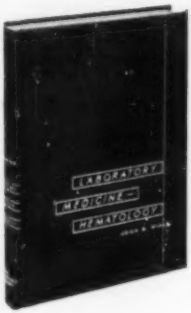

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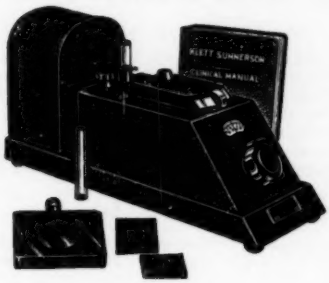

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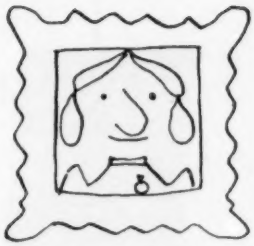

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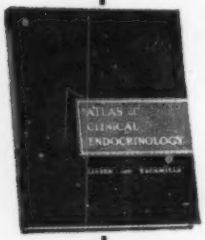

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Knickerbocker Biosales, Inc. ---------- 37

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Leitz, Inc., E. ---------- 3

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Ortho Pharmaceutical Corporation ---- 5

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Regent Scientific Co., Inc. ---------- 29
Rolfsmeyer Co., Dan (Rats and Mice) 33

Sanborn Company ---------- 6
Sargent & Company, E. H. ---------- 22
Schettle Frog Farm, Inc., J. R. ------ 31
Scientific Products, Div. of American Hospital Supply Corporation ------- 10
Standard Scientific Supply Corp. ------ 14
Steinhilber & Co., Inc., E. G. (Frogs) 32
Stocker, J. E. (White Mice, Guinea Pigs, and Rabbits) ---------- 31

Taconic Farms, Inc. (Swiss Mice) ---- 33
Technicon Instruments Corporation --- ---------- Third Cover
Thomas Company, Arthur H. -------- 24
Thomas, Charles C ---------- 18

Unitron, Instrument Div. of United Scientific Co. ---------- 15

Veeder-Root, Incorporated ---------- 29
Warner-Chilcott Laboratories ------- 2, 25
Worthington Biochemical Corp. ------ 28

Zeimet Bio-Farms (Guinea Pigs) ---- 32

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CONTENTS—*(Continued from Second Cover)*

The Effect of Insulin on the Hepatic Output of Glucose in the Dog. F. I. R. Martin, M.D., Jack R. Leonards, M.D., James W. Craig, M.D., Frank Barry, M.D., Max Miller, M.D., J. Ashmore, M.D., and W. Shoemaker, M.D., Cleveland, Ohio ---- 921

Potential Recovery of the Atrophic Kidney in Experimental Unilateral Renal Artery Stenosis. G. M. C. Masson, Ph.D., and T. Omae, M.D., Cleveland, Ohio ---- 922

Elevations of Serum Cholesterol in Man in Association With Life Stress and Independent of Diet and Exercise. William McCabe, M.D., James Hammarsten, M.D., William Schottstaedt, M.D., C. A. Adsett, M.D., Joe Yamamoto, M.D., and Stewart Wolf, M.D., Oklahoma City, Okla. ---- 922

The Effects of Serotonin on Segmental Coronary Vascular Resistance. William P. McKeever, M.D., Joseph D. Croft, Jr., B.A., and Donald M. Coder, B.A., Chicago, Ill. ---- 923

An Intensive Day-By-Day Study of Minor Respiratory Disease in Ten "Healthy" Adults. Michael McNamara, M.D., Eleanor Thomas, B.S., Annette Strobl, B.A., and Edwin D. Kilbourne, M.D., New York, N. Y. ---- 924

Regulatory Effect of Thyroid Hormone on Cortisol Metabolism. James C. Melby, M.D., Minneapolis, Minn. ---- 924

Studies on the Metabolism of Kidney Transplants. Benjamin F. Miller, Chem. Eng., M.D., Paul Nathan, Ph.D., E. C. Foulkes, Ph.D., and L. J. Wilchins, M.D., Cincinnati, Ohio ---- 925

Observations on the Site of Intestinal Absorption of Cobalt^{60}-Labeled Vitamin B_{12} in the Male Albino Rat. Charles G. Moertel, M.D., Harold H. Scudamore, M.D., Charles A. Owen, Jr., M.D., and Jesse L. Bollman, M.D., Rochester, Minn. ---- 926

The Measurement of Central Circulating Blood Volume From Precordial Dilution Curves in Normal Subjects and Patients With Mitral Stenosis. T. W. Moir, M.D., and F. S. Gott, B.S., Cleveland, Ohio ---- 926

Pyruvate Tolerance Tests in Healthy Subjects and in Patients With Diabetes Mellitus. John A. Moorhouse, M.D., Winnipeg, Manitoba ---- 927

Renal Hemodynamics in Unilateral and Bilateral Renal Artery Occlusive Disease and Response to Arterial Revascularization. George C. Morris, Jr., M.D., William B. Berry, B.S., Joseph R. Feste, B.S., and Michael E. De Bakey, M.D., Houston, Texas ---- 928

Enzymatic Activity Within the Human Hepatic Lobule. George Morrison, M.D., Irene Karl, Ph.D., Ruth Schwartz, Ph.D., and Robert E. Shank, M.D., St. Louis, Mo. 928

The Effect of Desoxypyridoxine Induced Vitamin B_6 Deficiency on Polyunsaturated Fatty Acid Metabolism in Human Beings. John F. Mueller, M.D., Jack Iacono, Ph.D., John J. Will, M.D., and William Repasky, M.D., Cincinnati, Ohio ---- 929

Antihypertensive Property of Extract From Renal Medulla. E. E. Muirhead, M.D., F. Jones, M.S., and J. A. Stirman, M.D., Dallas, Texas ---- 930

Detection and Assessment of Mitral Regurgitation Using Left Atrial Dilution Curves. C. Patrick Newcombe, M.D., John D. Sinclair, M.D., David E. Donald, D.V.M., Ph.D., and Earl H. Wood, M.D., Ph.D., Rochester, Minn. ---- 930

Adenosine Triphosphate in Human Liver. James F. O'Donnell, Ph.D., and Leon Schiff, M.D., Cincinnati, Ohio ---- 931

Butter, Corn Oil, and Fibrinolysis in Rats. R. M. O'Neal, M.D., W. A. Thomas, M.D., and R. W. Tillman, B.S., St. Louis, Mo. ---- 932

"Psychosomatic" Disease in Physicians: An Anterospective Study. Adrian M. Ostfeld, M.D., Joel S. Handler, M.D., and Buford Hall, M.D., Chicago, Ill. ---- 932

The Effect of 3-(3-Oxo-7 Alpha-Acetylthio-17 Beta-Hydrony-4-Androsten-17 Alpha-Yl)-Proprionic Acid Lactone (SC-9420) on Edematous Cardiac Patients. Robert G. Page, M.D., and A. R. Lavender, M.D., Chicago, Ill. ---- 933

The Effect of Granulocytotoxic Drugs Upo.. the Influx of S^{35} L-Cystine and L-Methionine Into Human Leukocytes. Anthony V. Pisciotta, M.D., Shirley N. Ebbe, M.D., Mona Ruwaldt, M.D., Mary Daly, B.S., Milton Glaser, M.D., and Gloria Metzger, M.S., Milwaukee, Wis. ---- 933

Lupus Glomerulonephritis: The Effect of Two Therapeutic Regimens on Renal Function and Renal Histology. Victor E. Pollak, M.B., M.R.C.P.(E.), Conrad L. Pirani, M.D., Robert C. Muehrcke, M.D., and Robert M. Kark, M.D., Chicago, Ill. ---- 934

(Continued on page 41)

CONTENTS—*(Continued from page 40)*

Hemolysate Tests Applied to the Study of the Hageman Trait. Armand J. Quick, M.D., and Clara V. Hussey, M.S., Milwaukee, Wis. ---- 935

The Natural Occurrence of Hemolytic Streptococci in Normal School Children (A Five-Year Study). Robert W. Quinn, M.D., and Margaret P. Martin, Ph.D., Nashville, Tenn. ---- 935

Ecologic Mental Illness—Psychiatry Exteriorized. Theron G. Randolph, M.D., Chicago, Ill. ---- 936

Mechanism of Growth Failure in Rats With Lesions of the Hypothalamus. Seymour Reichlin, M.D., Ph.D., St. Louis, Mo. ---- 937

The Mechanism of Action of Growth Hormone and Hydrocortisone on Protein Synthesis in Striated Muscles. Eric Reiss, M.D., and David Kipnis, M.D., St. Louis, Mo. ---- 937

The Modification of a Macrocryogelglobulin by Penicillin, Penicillamine, and Cysteamine. Stephan E. Ritzmann, M.D., Solon L. Coleman, M.D., and William C. Levin, M.D., Galveston, Texas ---- 938

A Method for Evaluation of Changes in Stroke Output and Vascular Resistance by Analysis of the Arterial Pulse. Simon Rodbard, M.D., Ph.D., Buffalo, N. Y. 939

Correlation of Electrocardiographic and Anatomic Findings in Right Ventricular Enlargement. Gonzolo T. Roman, Jr., M.D., Thomas J. Walsh, M.D., and Edward Massie, M.D., St. Louis, Mo. ---- 939

The Mechanism of Shunt Production in Atrial Septal Defect. G. G. Rowe, M.D., J. E. Clifford, M.D., C. A. Castillo, M.D., G. M. Maxwell, M.D., and C. W. Crumpton, M.D., Madison, Wis. ---- 940

Effects of a Chemical Inhibitor of 11-B Hydroxylation on the Urinary Steroid Pattern in Primary Hyperaldosteronism. Robert M. Salassa, M.D., and Vernon R. Mattox, Ph.D., Rochester, Minn. ---- 940

Studies With Pure Decapeptide Angiotensin. I. General and Pulmonary Hemodynamics. Salvatore M. Sancetta, M.D., Cleveland, Ohio ---- 941

Studies on the Evaluation of Tularemia Vaccines in Man. Samuel Saslaw, M.D., Ph.D., Henry E. Wilson, M.D., John A. Prior, M.D., and Sally R. Carhart, M.S., Columbus, Ohio ---- 941

A Unified Approach to Uneven Distribution of Blood and Gas in the Lungs. George A. Saxton, Jr., Chicago, Ill. ---- 942

Effect of Serotonin and Histamine on Connective Tissue in the Rat. Arthur L. Scherbel, M.D., Roy L. McKittrick, M.D., and William A. Hawk, M.D., Cleveland, Ohio 943

The Distribution of Blood Flow in the Kidney of the Dog. James F. Schieve, M.D., James W. Carhart, M.D., Robert N. Watman, M.D., and Richard W. Stow, Ph.D., Columbus, Ohio ---- 943

The Hypoglycemic Effect of L-Leucine in Organic Hyperinsulinism. Theodore B. Schwartz, M.D., George C. Flanagan, M.D., George W. Stuppy, M.D., and Donald W. Tarum, M.D., Chicago, Ill. ---- 944

Lack of Immunity Following Tetanus. Paul M. Seebohm, M.D., Albert P. McKee, M.D., and Wayburn S. Jeter, Ph.D., Iowa City, Iowa ---- 945

Circulating Insulin Activity After Glucose: Relation of Insulogenic Reserve to Clinical Severity of Diabetes Mellitus. Holbrooke S. Seltzer, M.D., and Walter L. Smith, M.S., Dallas, Texas ---- 945

A Study of Adrenal Steroids in Blood and Urine in Acute and Chronic Rheumatic Fever. Earl N. Silber, M.D., Paul Salzman, M.D., Aaron Kerlow, M.D., and Jay J. Gold, M.D., Chicago, Ill. ---- 946

Tryptic Digestion Interfered With by Asthmatic Sputum. Jeanne M[illegible]tgomery Smith, M.D., Iowa City, Iowa ---- 947

Comparative Assays of Purified Sheep FSH and Human Pituitary Gonadotropins. Richmond W. Smith, Jr., M.D., Raymond C. Mellinger, M.D., Jalileh Mansour, and Irene T. Kline, Ph.D., Detroit, Mich. ---- 947

Epidemiologic Analysis of Patterns of Blood Pressure and Weight Evolution, and Hypertensive and Atherosclerotic Disease Development—30-Year Follow-up Data on the Labor Force of a Chicago Utility Company. Jeremiah Stamler, M.D., Howard A. Lindberg, M.D., David M. Berkson, M.D., and Wilda Miller, B.S., Chicago, Ill. 948

(Continued on page 42)

CONTENTS—*(Continued from page 41)*

The Danger of ACTH in Addison's Disease. Daniel B. Stone, M.B., and Judith G. Jewel, M.D., Iowa City, Iowa ---- 949

Studies on the Pathogenesis of Idiopathic Edema. David H. P. Streeten, M.R.C.P., D.Phil., and Jerome W. Conn, M.D., Ann Arbor, Mich. ---- 949

Zinc Excretion in Chronic Alcoholism and Laennec's Cirrhosis. James F. Sullivan, M.D., and Kathleen T. Hart, Ph.D., St. Louis, Mo. ---- 950

Studies of Renin and Juxtaglomerular Cells, Components of a Regulating System in the Kidney. Louis Tobian, M.D., Minneapolis, Minn. ---- 951

The Influence of Dietary Fats on the Fatty Acids of Blood, Atheromatous Aorta, Liver, and Depot Fat in Rabbits. Naip Tuna, M.D., Ph.D., Mary J. Sundeen, B.S., and Mary L. Louden, B.A., Minneapolis, Minn. ---- 951

Production of Glucagon Antibodies and the Development of an Immunoassay for Glucagon. Roger H. Unger, M.D., Anna M. Eisentraut, Mary S. McCall, B.S., Sue Keller, B.S., and Leonard L. Madison, M.D., Dallas, Texas ---- 952

Pancreatic Function in Cirrhosis of the Liver. Joseph B. Vacca, M.D., Wilbert J. Henke, M.D., and William A. Knight, Jr., M.D., St. Louis, Mo. ---- 952

The Pathogenesis and Pathology of Anaphylactoid (Henoch-Schönlein's) Purpura. Robert L. Vernier, M.D., Howard G. Worthen, M.D., and Eleanor Colle, M.D., Minneapolis, Minn. ---- 953

The Effect of Hyperthyroidism on Skeletal Muscle Metabolism. P. J. Vignos, Jr., M.D., and L. Lapham, M.D., Cleveland, Ohio ---- 954

A Human Standard Clot: Observations and Measurement on Its Induced Disintegration. K. N. von Kaulla, M.D., Denver, Colo. ---- 955

The Bactericidal Activity of Human Sera Against Escherichia Coli. Burton A. Waisbren, M.D., and Imogene Brown, M.S., Milwaukee, Wis. ---- 955

Phenylalanine Tolerance Tests in Patients With Leukemia. Harry A. Waisman, M.D., Ph.D., Hwa Lih Wang, Ph.D., Madison, Wis. ---- 956

The Mechanism of Hemorrhage in Thrombocytosis. Robert L. Wall, M.D., Columbus, Ohio ---- 956

Stroke Volume in Man During Exercise. Yang Wang, M.D., Robert J. Marshall, M.D., and John T. Sheperd, M.D., Rochester, Minn. ---- 957

Epicardial Potentials in Right Ventricular Hypertrophy. R. H. Wasserburger, M.D., W. P. Young, M.D., K. Siebecker, Jr., M.D., and T. J. King, M.D., Madison, Wis. 958

Abnormal Calcium-Binding Associated With Hyperglobulinemia, Clotting Defects, and Osteoporosis: A Study of This Relationship. Leo Wayne, M.D., Richard E. Goldsmith, M.D., Helen I. Glueck, M.D., and Helen K. Berry, Cincinnati, Ohio 958

Evidence Suggesting Differences Between Bactericidal and Hemolytic Complement. Ralph J. Wedgwood, M.D., Cleveland, Ohio ---- 959

Defective Thiamine Utilization in Patients Having Chronic Neurologic Disease. David R. Weir, M.D., Cleveland, Ohio ---- 960

The Thromboplastic Activity of Soybean Phospholipids. William J. Williams, M.D., Philadelphia, Pa. ---- 960

The Coincidence of Leukocyte and Thrombocyte Antibodies. Henry E. Wilson, M.D., Susan A. Naegeli, B.S., and Ali Hossaini, B.S., Columbus, Ohio ---- 961

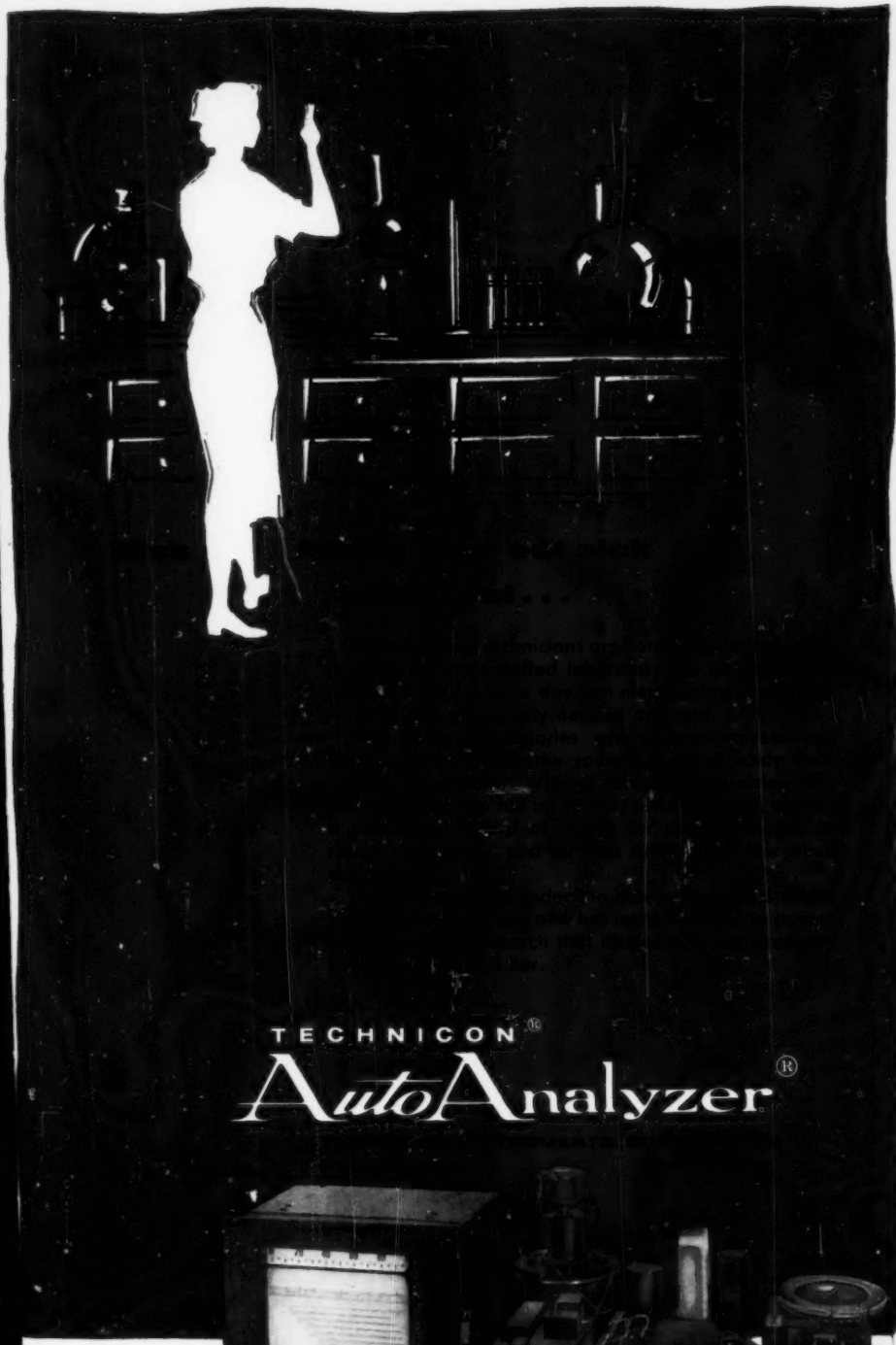
A "Successful" Staphylococcal Control Program Which Failed. Robert E. Windom, M.D., Jay P. Sanford, M.D., Richard A. Fergeson, and Chester W. Fink, M.D., Dallas, Texas ---- 962

The Effect of Prior Sensitization With Freund Adjuvant Upon Experimental Allergic Encephalomyelitis. Walter S. Wood, M.D., Chicago, Ill. ---- 962

Lipids and Lipid Mobilizer Hormone in Pregnancy and the Newborn Infant. Chris J. D. Zarafonetis, M.D., Joseph Seifter, M.D., David H. Baeder, Ph.D., and John P. Kalas, M.D., Philadelphia, Pa. ---- 963

Index

Index ---- 967



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